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Evaluation of certain food additives

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

Food and Agriculture Organization of the United Nations

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This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the World Health Organization

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Contents

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

Geneva, 17–26 June 2014

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List of abbreviations

ADI	acceptable daily intake
bw	body weight
CAS	Chemical Abstracts Service
CCCF	Codex Committee on Contaminants in Foods
CCFA	Codex Committee on Food Additives
CITREM	citric and fatty acid esters of glycerol
DNA	deoxyribonucleic acid
EFSA	European Food Safety Authority
Eq	equivalents
EU-16	Austria, Belgium, Cyprus, Finland, France, Germany, Greece, Ireland, Italy, Latvia, Luxembourg, Malta, Portugal, Slovakia, Slovenia and Spain
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association of the United States
GLP	good laboratory practice
GRAS	Generally Recognized as Safe
GSFA	(Codex) General Standard for Food Additives
HLB	hydrophilic–lipophilic balance
IL	interleukin
INS	International Numbering System for Food Additives
IOFI	International Organization of the Flavor Industry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
ML	maximum level
MOE	margin of exposure
MSDI	maximized survey-derived intake
NMR	nuclear magnetic resonance
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
OSA	octenyl succinic acid
pAOS	pectin-derived acidic oligosaccharides
PAS	potassium aluminium silicate
SPET	single-portion exposure technique
TNF	tumour necrosis factor
TTC	threshold of toxicological concern
USA	United States of America
WHO	World Health Organization
w/w	weight per weight

Dedication

Dr John Christian Larsen

National Food Institute, Technical University of Denmark (retired)

It was with great sadness that the Committee noted the passing of Dr John Christian Larsen. John Christian served on the Committee since the early 1990s in various capacities, including as Rapporteur and as Chair. He chaired many meetings, and in his unique, quiet and thoughtful way, he led the Committee to successful outcomes. With his leadership and breadth of scientific knowledge, he guided the Committee through discussions that at times were difficult and controversial. He brought not only dedication and thoroughness to the work of the Committee, but also a human touch. John Christian will be thoroughly missed by his peers and friends in the scientific community.

In recognition of his services, the Committee dedicated this report to the memory of Dr John Christian Larsen.

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. 70, 2014.

Specifications are issued separately by FAO under the title:

Compendium of food additive specifications. FAO JECFA Monographs 16, 2014.

1. Introduction

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) met in Geneva from 17 to 26 June 2014. The meeting was opened by Dr Kazuaki Miyagishima, Director of the Department of Food Safety and Zoonoses of the World Health Organization (WHO), who welcomed participants on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations (FAO) and WHO. Dr Miyagishima commented that JECFA was one of the most successful joint undertakings of FAO and WHO, playing a critical role in the development of international food safety standards by the Codex Alimentarius Commission. He noted that although the integrity and quality of the work of JECFA are sometimes questioned by the media or activist groups, WHO and FAO will defend the Committee or individual participants who are unjustly attacked.

Dr Miyagishima mentioned several ongoing initiatives and discussions of relevance to food safety. The Millennium Development Goals will be coming to the end of their term in 2015, and the United Nations is preparing to set a series of new targets to support sustainable development worldwide. The work of JECFA plays an important role in the provision of safe, affordable and nutritious food – one of the focus areas in the discussion on the sustainable development goals of the United Nations – by providing the scientific basis for policy decisions. An international conference on nutrition (ICN2), to be held in Rome in November 2014, will include some discussion on food safety, and Milan will host the World Expo 2015 with a focus on food, which will be another opportunity to highlight international work on food safety.

One of the tasks before this Committee is to evaluate the safety of four food additives for specific use in infant formulas, as requested by the Codex Committee on Food Additives (CCFA). Another task is the evaluation of several plant extracts for use as colours, which presents a unique challenge in terms of ensuring that the products in commerce are clearly defined and safe for their intended uses. One of the important general considerations on the agenda is related to the application of the principle of the threshold of toxicological concern (TTC) for the evaluation of flavouring agents. Another important general consideration relates to the limit of lead in the specifications for food additives intended for use in infant foods.

Dr Miyagishima reminded participants that they have been invited to this meeting as independent experts and not as representatives of their countries or organizations. He also reminded them of the confidential nature of this meeting, which allows experts to freely express their opinions. He closed by expressing his sincere gratitude to participants for providing their time and expertise to this important work, which contributes to the core business of both FAO and WHO, providing science-based international norms and standards.

1.1 Declarations of interests

The Secretariat informed the Committee that all experts participating in the seventy-ninth meeting had completed declaration of interest forms and that no conflicts of interest were identified. The following declared interests and potential conflicts were discussed by the Committee. Data on gardenia yellow and polyoxyethylene (20) sorbitan monostearate were submitted from the institution where Dr Yoko Kawamura is working, although she was not involved in the preparation of the data submission and was not assigned to the evaluation of these substances. Professor Gary Williams' research group received a research grant through his university from the International Organization of the Flavor Industry (IOFI) to investigate the toxicological mode of action for a flavour compound not on the agenda; and he served on the Generally Recognized as Safe (GRAS) panel for Intertek for the evaluation of polysorbates (on the agenda of this meeting for revision of specifications only). Professor Glenn Sipes participated on several GRAS panels, none of them on any compounds on the agenda. He also reviewed a document on octenyl succinic acid (OSA)–modified starch for Intertek. He was not aware if this work was related to the submission, but he did not participate in the discussion on this compound. Dr Josef Schlatter served on an expert panel of the Flavor and Extract Manufacturers Association of the United States (FEMA) that discussed three flavouring compounds on the agenda of this meeting. He did not participate in discussions on these flavouring agents at this meeting.

1.2 Modification of the agenda

The Committee made the following modifications to the agenda (see original agenda in Annex 4):

- Following the request from CCFA to consider the deletion of the functional use of carrier from the specifications for potassium aluminium silicate (PAS) (INS 555), the Committee agreed to add this item under agenda item 7.2 (food additives for revision of specifications only).
- *Tagetes* extract was on the agenda at the request of CCFA to undertake a safety assessment and revision of specifications. The Committee noted that the information supplied by the sponsor referred to a substance of a higher carotenoid ester content; therefore, it was decided to name it "Lutein esters from *Tagetes erecta*" to differentiate it from *Tagetes* extract.
- The Committee determined that the flavouring agent α-ionene (No. 2193) did not belong in the flavouring agent group of aliphatic and alicyclic hydrocarbons and did not evaluate it at the meeting.

- The Committee was able to perform a safety evaluation of Benzoe tonkinensis, so it was moved from agenda item 7.2 (food additives for revision of specifications only) to agenda item 7.1 (toxicological evaluations).

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 78 previous meetings of the Committee (Annex 1). The present meeting was convened on the basis of a recommendation made at the seventy-seventh meeting (Annex 1, reference *214*).

The tasks before the Committee were to:

- elaborate further principles for evaluating the safety of food additives (including flavouring agents) (section 2);
- review and prepare specifications for certain food additives (including flavouring agents) (section 3 and Annex 2);
- undertake safety evaluations of certain food additives (including flavouring agents) (sections 3 and 4 and Annex 2).

2.1 Report from the Forty-sixth Session of the Codex Committee on Food Additives (CCFA)

The Codex Secretariat provided the Committee with an update on the work of CCFA since the seventy-seventh meeting of JECFA (Annex 1, reference *214*).

Following the seventy-seventh meeting of JECFA, the Forty-sixth Session of CCFA *(2)* agreed to wait for further evaluation of advantame (INS 969) and OSA-modified gum arabic (INS 423) and removed Note 28 on nisin acceptable daily intake (ADI) conversion from the Codex General Standard for Food Additives (GSFA) *(3)*. It also recommended the inclusion of glucoamylase from *Trichoderma reesei* expressed in *Trichoderma reesei* in the database on processing aids prepared by China (www.ccfa.cc/IPA) and noted that no action was required for glycerol ester of tall oil rosin (INS 445(i)) or glycerol ester of wood rosin (INS 445(ii)).

Work on more than 700 provisions of the GSFA was finalized, and the adoption of new and revised specifications for the identity and purity of 10 food additives, prepared by the seventy-seventh meeting of JECFA, was recommended. With regard to PAS (INS 555), CCFA requested JECFA to consider the deletion of the functional use of carrier from the specifications.

The Forty-sixth Session of CCFA agreed on a revised priority list of substances for evaluation (or re-evaluation) by JECFA and assigned high priority to eight of them. CCFA also included benzoates on the priority list for exposure assessment. With regard to the food additives included in the GSFA for which there were no corresponding JECFA specifications, CCFA removed from the GSFA the food additives for which no information on their commercial use was provided and included the others on the priority list subject to confirmation of

the provision of dossiers by its next session. With regard to the re-evaluation of substances, CCFA agreed to a process on a trial basis, which would use six prioritized food colours as a working example. These colours will be added to the priority list, under a separate table, subject to confirmation of the availability of data to the next session of CCFA.

The Forty-sixth Session of CCFA completed work on the alignment of the food additive provisions in five standards for meat products and the corresponding provisions of the GSFA and agreed to continue working on the food additive provisions of other standards. Work on alignment will result in the GSFA becoming the only reference for food additives in Codex. Only additives evaluated by JECFA can be included in the GSFA.

The Forty-sixth Session of CCFA completed work on the Guidelines for the Simple Evaluation of Dietary Exposure to Food Additives *(4)*. The Guidelines are intended to facilitate the work of countries with limited resources on the assessment of dietary exposure to food additives. CCFA further agreed to consider at its next session a document on secondary additives, which will address a definition and analyse the issues and potential inconsistencies in the CCFA work.

2.2 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 *(5)*.

2.3 Threshold of toxicological concern (TTC) principle: update on a WHO project and implications for the Procedure for the Safety Evaluation of Flavouring Agents

The Committee was informed about a project that WHO is undertaking in collaboration with the European Food Safety Authority (EFSA) on a review of the application of the TTC principle in the risk assessment of chemicals, based on the current state-of-the-science and building on existing work.

A draft report was presented reviewing the Cramer classification scheme *(6)*, with a focus on how metabolism is taken into account and a review of class thresholds and the underlying science.

A revised JECFA decision-tree for the evaluation of flavours was proposed. After a brief discussion, the Committee recommended that further considerations are necessary and that a proposal should be drafted for consideration at the next JECFA meeting at which flavouring agents will be evaluated.

The Committee was also informed about a new decision-tree under development by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) for the evaluation of pesticide metabolites using the TTC principle.

2.4 Food additive specifications

2.4.1 Limits for lead in specifications of food additives for use in infant formulas

The Committee at the present meeting considered four additives for use in infant formula and formula for special medical purposes – namely, carrageenan; citric and fatty acid esters of glycerol (CITREM); pectin; and starch sodium octenyl succinate (OSA-modified starch). The Committee noted that the Eighth Session of the Codex Committee on Contaminants in Foods (CCCF) *(7)* agreed to a maximum level (ML) of 0.01 mg/kg for lead in infant formula (as consumed). The Committee also noted that with the exception of carrageenan, use of the other three food additives at proposed use levels could result in an exceedance of the ML of lead in infant formula. This situation was estimated to occur if lead were present in the additive at the specified limit – i.e. 2 mg/kg in CITREM and starch sodium octenyl succinate (OSA-modified starch) and 5 mg/kg in pectin. This estimation was calculated without considering the contribution of other ingredients to the overall lead level in infant formulas.

The Committee noted that the responsibility for ensuring that the final infant formulas comply with the ML for lead remains with infant formula producers. Furthermore, the Committee noted that data provided at the present meeting by the sponsors indicate that individual food additives can be produced with lead levels below the specified limits as listed above. Considering this, the Committee noted that lower lead limits in the specifications – for instance, 0.1 mg/kg for starch sodium octenyl succinate (OSA-modified starch), 1 mg/kg for pectin and 0.5 mg/kg for CITREM – would result in none of the additives exceeding the ML for lead in the final infant formula (i.e. 0.01 mg/kg). The specifications for some of the food additives for use in infant formulas that were considered for safety review at this meeting are also used in the manufacture of other foods. Thus, the Committee agreed that it would be necessary to confirm with manufacturers that the lower lead limits would also be achievable for the intended use of these food additives in products other than infant formulas.

The Committee refers back to CCFA on whether specific purity criteria for additives for use in infant formulas should be considered and appropriate ways to present these criteria (e.g. establishing specifications for additives for use in infant formulas only; establishing different purity limits for additives for use in existing specifications).

As an additional consideration, the Committee noted that if separate specifications for additives in infant formulas were considered necessary, microbiological criteria should also be included.

2.4.2 Method for alginates assay

While reviewing the specifications for gellan gum, the Committee noted that the method of assay referred to the alginates assay method in Volume 4 of the Combined Compendium of Food Additive Specifications (Annex 1, reference *180*), which uses mercury as one of its reagents. The Committee replaced the alginates assay method with a new method, based on the United States Pharmacopeia *(8)*, without the use of mercury. The revised method will be published in the Compendium of Food Additive Specifications, FAO JECFA Monographs 16 (2014).

The online version of the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1, Volume 4, will also be revised.

2.4.3 Oxalate limit test

While reviewing the specifications for citric acid, the Committee recognized that the oxalic acid standard used for comparison in the oxalate limit test, as described in Volume 4 of the Combined Compendium of Food Additive Specifications (Annex 1, reference *180*), does not represent the limit specified in the individual specifications monographs for different food additives. The method was revised to use an appropriate standard solution that represents the limit specified. The revised test method will be published in the Compendium of Food Additive Specifications, FAO JECFA Monographs 16 (2014).

The online version of the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1, Volume 4, will also be revised.

2.5 The use of the margin of exposure (MOE) for the evaluation of additives used in infant formulas

The ADI concept does not apply to infants up to the age of 12 weeks because they might be at risk at lower levels of exposure compared with older age groups. This is due to special considerations, such as their immature metabolic capacities, the greater permeability of the immature gut, and their rapid growth and development. Therefore, risk characterization for very young infants has to be considered on a case-by-case basis.

Toxicological testing strategies for additives to be used in infant formulas require approaches that differ from those generally adopted for food additives. For example, evaluation of food additives to be used in infant formulas requires

consideration of safety studies involving exposure of very young animals *(5)*. The reproductive and developmental toxicity studies commonly available for evaluations of chemicals in food address the possible impact on neonatal animals arising through in utero and lactational exposure. However, they frequently do not incorporate direct oral administration to neonatal animals, and such studies are required for the evaluation of food additives in infant formula. If the additive is proposed for use in infant formula at relatively high levels (e.g. 0.1% or greater), then conducting toxicological studies in neonatal animals at doses two or more orders of magnitude greater than the anticipated human exposure, which is the approach commonly taken for food additives, may not be feasible.

The Committee noted that for three of the four food additives on its current agenda that are proposed for use in infant formulas, the margins of exposure (MOEs) between the no-observed-adverse-effect level (NOAEL) and the estimated daily exposures to the food additives were in the range of 0.8–12 for infants. Interpretation of the MOE needs to take into account uncertainties or conservatisms that may exist in the toxicological point of departure or in the exposure estimates.

Considerations related to the toxicological point of departure to be taken into account in interpreting the MOE include:

- absorption, distribution, metabolism and excretion – for example, whether or not the additive is absorbed, comparison of potential for metabolic activation and detoxication in the neonatal organism compared with the adult;
- the overall toxicological profile of the substance, including identification of critical effects;
- the potential effects of exposure during life stages in experimental animals of relevance to human infants;
- the relevance for the human infant of the neonatal animal models used in toxicological testing;
- whether adverse effects have been identified in the toxicological studies in neonatal animals, or if the NOAELs are the highest doses tested;
- the design and outcome of any clinical studies conducted with infants (e.g. total number and age of infants tested, growth, tolerance, types of adverse reaction examined); and
- reports of adverse reactions in post-marketing surveillance, where the infant formula is already in use in some countries.

Factors related to the dietary exposure assessments that should be taken into account for the interpretation of an MOE include the following assumptions and considerations:

- Formula is the only source of nutrition for the first 12 weeks of life.
- The additive will be used at the maximum proposed level.
- An energy density of 67 kcal/100 mL (280 kJ/100 mL) is used to convert energy to the volume of formula ingested daily.
- High infant formula consumption is derived from 95th percentile energy intakes.
- Variability of exposure among infants is small.
- Duration of exposure is for a limited time, and exposure decreases on a body weight basis during the exposure period.

The Committee concluded that when the above issues have been taken into account, an MOE in the region of 1–10 could be interpreted as indicating low risk for the health of infants aged 0–12 weeks consuming the food additive in infant formula.

2.6 Need for an approach for prioritizing flavouring agents for re-evaluation

At this meeting, the Committee held a preliminary discussion concerning the fact that the submission of additional toxicology data, including genotoxicity data, and/or exposure data for new or previously evaluated flavouring agents may trigger the need for re-evaluation of previously evaluated flavouring agents.

Three examples encountered at the present meeting are described. In the first example, 3-acetyl-2,5-dimethylthiophene (No. 1051) was on the agenda for reconsideration at this meeting because new data suggested genotoxic potential. Positive in vitro and in vivo genotoxicity data raised concerns about No. 1051 and previously evaluated thiophenes that are metabolized to thiophene epoxides, indicating that reconsideration of the Committee's conclusions regarding the safety of the previously evaluated thiophenes is warranted.

Second, 2-phenyl-2-butenal (No. 1474) was evaluated earlier as a flavouring agent, and it is structurally related to (±)-2-phenyl-4-methyl-2-hexenal (No. 2069), under consideration at this meeting. Genotoxicity data for No. 1474, used as a structural analogue for No. 2069, were equivocal, raising concerns about the potential genotoxicity of No. 1474 and possibly other previously evaluated compounds with similar structures in this group, in addition to No. 2069. The Committee noted that No. 2069 should not be evaluated for use as a flavouring agent until the concerns related to genotoxicity are resolved, and the safe use

of No. 1474 and structurally related substances as flavouring agents should be reconsidered.

Third, *trans*-α-damascone (No. 2188) was submitted for evaluation at the current meeting of the Committee. Several isomers of No. 2188 were evaluated previously by the Committee, including β-damascone (No. 384), α-damascone (No. 385) and δ-damascone (No. 386), and each was found to be of no safety concern based on dietary exposures estimated by the maximized survey-derived intake (MSDI) method. At this meeting, the same toxicological database used for the evaluation of Nos 384–386 was used for No. 2188. However, the NOAEL for No. 384, used as a structural analogue for No. 2188, was only 200 times the single-portion exposure technique (SPET) estimate for exposure to No. 2188 (600 μg/day). If the SPET estimate of exposure for use of No. 384, No. 385 or No. 386 as a flavouring agent is similar to that for No. 2188, the safety of each of these compounds for use as a flavouring agent could be called into question.

Based on the evaluations conducted on these flavouring agents at the present meeting, the Committee recommended that an approach be developed for prioritizing flavouring agents for re-evaluation based on all available toxicological data and updated exposure estimates.

3. Specific food additives (other than flavouring agents)

The Committee evaluated two food additives for the first time and re-evaluated three others. In addition, the Committee evaluated the safety of four previously evaluated food additives for use in infant formula and formula for special medical purposes intended for infants. Five 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 section 5.

3.1 Safety evaluations

3.1.1 Benzoe tonkinensis

Explanation

Benzoe tonkinensis is a balsamic resin from the *Styrax tonkinensis* Pierre Craib ex Hartwich tree, which belongs to the Styracaceae family. Benzoe tonkinensis is being evaluated for use as a flavouring agent. The Committee previously considered benzoin gum at its twenty-first, fifty-fifth and seventy-fourth meetings (Annex 1, references *44*, *149* and *205*). At its twenty-first meeting, the Committee prepared tentative specifications covering two forms of benzoin gum (Benzoe tonkinensis and Sumatra benzoin). However, no ADI was established, and no monograph was prepared. At its fifty-fifth meeting, the Committee withdrew the tentative specifications for benzoin gum, as the relevant information was not provided. At its seventy-fourth meeting, the Committee evaluated new information on the composition and the toxicity of Benzoe tonkinensis. Tentative specifications, a Chemical and Technical Assessment and a toxicological monograph were prepared. Comparing the dietary exposure estimate (95th percentile for children) for Benzoe tonkinensis of 0.2 mg/kg body weight (bw) per day with the NOAEL of 500 mg/kg bw per day identified in a 90-day oral toxicity study in rats, the MOE was at least 2500. At the seventy-fourth meeting, the Committee noted that exposure to benzoic acid and benzyl benzoate from the use of Benzoe tonkinensis was well below the upper limit of the group ADI (0–5 mg/kg bw) for benzyl derivatives and that exposure to vanillin was also well below the upper limit of its ADI (0–10 mg/kg bw).

At the seventy-seventh meeting (Annex 1, reference *214*), the Committee revised the specifications, and the tentative status was maintained pending submission of data on microbiological contamination, composition of the ethanolic extract and an analytical method to distinguish between Benzoe tonkinensis and Sumatra benzoin. At the current meeting, additional compositional data were evaluated, and the Committee was able to finalize the safety evaluation.

Chemical and technical considerations

The main compounds identified in Benzoe tonkinensis were benzoic acid, coniferyl benzoate, vanillin and benzyl benzoate. Other compounds, namely *p*-coumaryl benzoate, siaresinolic acid, 3-oxo-siaresinolic acid, sumaresinolic acid and 3-oxo-sumaresinolic acid, were also identified in minor amounts (<1% of the total).

Evaluation

New information on the composition of Benzoe tonkinensis clarified its main constituents. The Committee concluded that dietary exposure to the newly identified compounds (siaresinolic acid, sumaresinolic acid and the respective 3-oxo compounds) would be very low, as their concentrations in Benzoe tonkinensis are very low (<1% of the total).

Given the NOAEL of 500 mg/kg bw per day for Benzoe tonkinensis identified in a 90-day oral toxicity study in rats and the previously established ADIs for the major components, the Committee confirmed the conclusion from the seventy-fourth meeting that Benzoe tonkinensis would not be of safety concern at current estimated dietary exposures, provided that it complies with the specifications prepared at the current meeting, when used as a flavouring agent and in accordance with good manufacturing practice.

The Committee revised the tentative specifications, and the tentative qualification was removed. The definition was amended to identify the main compounds. Microbiological criteria regarding *Salmonella* spp., *Escherichia coli* and yeast and moulds were introduced. The gas chromatographic method for the determination of benzoic acid was updated to allow, besides the determination of benzoic acid and identification of vanillin and benzyl benzoate, the confirmation of the absence of cinnamic acid, which is a marker molecule to differentiate Benzoe tonkinensis from Sumatra benzoin. The existing Chemical and Technical Assessment was revised.

No toxicological monograph was prepared.

3.1.2 Carrageenan

Explanation

Carrageenan is a sulfated galactose polymer with an average molecular weight well above 100 kDa. It is derived from several species of red seaweeds of the class Rhodophyceae. The three main copolymers of carrageenan used in food are designated as iota (ι), kappa (κ) and lambda (λ), depending on the number and location of the sulfate moieties on the hexose backbone. Carrageenan has no nutritive value and is used in foods for its thickening, gelling, stabilizing and glazing agent properties.

Carrageenan was reviewed by the Committee at its thirteenth, seventeenth, twenty-eighth, fifty-first, fifty-seventh and sixty-eighth meetings (Annex 1, references *19*, *32*, *66*, *137*, *154* and *187*). At its twenty-eighth meeting, the Committee established an ADI "not specified" on the basis of the results of a number of toxicological studies on carrageenans obtained from various sources. Processed *Eucheuma* seaweed was reviewed by the Committee at its thirtieth, thirty-ninth, forty-first, forty-fourth, fifty-first and fifty-seventh meetings (Annex 1, references *73*, *101*, *107*, *116*, *137* and *154*). At its fifty-first meeting, the Committee concluded that the toxicities of processed *Eucheuma* seaweed and carrageenan were sufficiently similar for the ADI "not specified" for carrageenan to be extended to a temporary group ADI including processed *Eucheuma* seaweed, pending clarification of the significance of the tumour promotion of known experimental colon carcinogens by carrageenan observed in experiments in rats. At its fifty-seventh meeting, the Committee established a group ADI "not specified" for the sum of carrageenan and processed *Eucheuma* seaweed, as the Committee considered that the intakes of carrageenan and processed *Eucheuma* seaweed from their use as food additives were of no safety concern.

At its sixty-eighth meeting, the Committee reviewed all the available data for toxicological re-evaluation, including specific data relevant to the safety assessment for infants from exposure through infant formula. The previous Committee concluded that potential effects of carrageenan in infants could arise either from a direct action on the epithelium of the intestinal tract, which would be related to the concentration of carrageenan in infant formula, or from absorption of the low molecular weight fraction of carrageenan, which would be more likely to be related to the dietary exposure expressed on a body weight basis. Therefore, the previous Committee estimated MOEs for infants on the basis of both concentration and body weight. In the absence of studies on the immature gut, the Committee used data from studies on adult rodents, which identified the lowest doses causing inflammatory responses in the gut to be in the range 1100–1300 mg/kg bw per day, to derive MOEs. The MOE between the concentration in drinking-water reported to cause inflammation in adult mice and the maximum concentration (0.1%) of carrageenan in infant formula was 10. On a body weight basis, for preweaning infants at the maximum concentration (0.1%) of carrageenan in infant formula, the MOE between the lowest doses reported to cause inflammation in rats and mice and infant exposure was 7; for 12-month-old infants, the MOEs were 50 and 180 at carrageenan concentrations of 0.1% and 0.03%, respectively. The previous Committee considered these MOEs to be insufficient to ensure the protection of infants fed infant formula containing carrageenan and was therefore of the view that it is inadvisable to use carrageenan or processed *Eucheuma* seaweed in infant formula intended for infants up to and including 12 months of age. The group ADI "not specified" for

the sum of carrageenan and processed *Eucheuma* seaweed was maintained for food additive uses in foods other than infant formula.

At the present meeting, the Committee reviewed data published since the sixty-eighth meeting in 2007, focusing in particular on data of relevance to the safety assessment of the use of carrageenan (but not processed *Eucheuma* seaweed) in infant formula. The use levels requested for carrageenan range from 90 to 1000 mg/L, with 300 mg/L as a typical use level for a standard infant formula and higher levels up to 1000 mg/L for liquid formulas for special medical purposes containing hydrolysed protein or amino acids. In response to the Committee's request for further data, a toxicological dossier on carrageenan and a commentary on studies published by an academic research laboratory were submitted. The Committee also considered other information available in the literature of relevance to carrageenan and to the signalling pathways involved in inflammation.

Chemical and technical considerations

Carrageenan is a sulfated galactose polymer, the most important forms being κ-, ι- and λ-carrageenans. κ-Carrageenan (Chemical Abstracts Service [CAS] No. 11114-20-8) is mostly the alternating copolymer of D-galactose-4-sulfate and 3,6-anhydro-D-galactose. ι-Carrageenan (CAS No. 9062-07-1) is similar, except that the 3,6-anhydrogalactose is sulfated at carbon two. Between κ-carrageenan and ι-carrageenan, there is a continuum of intermediate compositions differing in degree of sulfation at carbon two. In λ-carrageenan (CAS No. 9064-57-7), the alternating monomeric units are mostly D-galactose-2-sulfate (1,3-linked) and D-galactose-2,6-disulfate (1,4-linked). Various red seaweeds are blended and processed to obtain the desired proportions of κ-, ι- and λ-carrageenan to satisfy food technological requirements. In addition to the polysaccharide components of carrageenan, the product also contains salts (usually potassium chloride to maintain the desired gelling properties) and sugars added for standardization purposes. Other carbohydrate residues (e.g. xylose, glucose and uronic acids) may be present in minor amounts.

Carrageenan has a high average molecular weight distribution of 200–800 kDa, with a small fraction containing naturally occurring fragments in the 20–50 kDa range. A survey of 29 samples of food-grade carrageenan representing κ-, ι- and λ-carrageenan determined a number average molecular weight of 193–324 kDa and a weight average molecular weight of 453–652 kDa. Food-grade carrageenan has a viscosity specification of not less than 5 cP at 75 °C (1.5% solution), which corresponds to an average molecular weight of approximately 100–150 kDa.

Poligeenan (CAS No. 53973-98-1) has been used in research studies as a surrogate for the low molecular weight fraction of carrageenan. It is produced under severe acid and high-temperature conditions and has an average molecular weight of 10–20 kDa. Poligeenan is distinct from food-grade carrageenan. Although the average molecular weights of carrageenan and poligeenan are significantly different, when the molecular weight distributions of the two substances are compared, there is a small overlap between the two at the lower molecular weight portion of the carrageenan distribution and the higher molecular weight portion of the poligeenan distribution, with the range of interest being between 20 and 50 kDa.

When carrageenan is present in water at less than about 0.1% with no dietary solids or protein, the carrageenan molecules are random open coils and available for maximum interaction with other molecules. κ-Carrageenan in the presence of potassium cations and ι-carrageenan in the presence of calcium cations will gel at concentrations above 0.1% in water if a heating/cooling cycle is applied. This requires heating to above about 60 °C, at which temperature the carrageenan is in a random open coil. On cooling to less than about 35 °C, the solutions will gel, forming double helices; then, for κ-carrageenan only, these helices will aggregate and form a more tightly closed structure. The transition between sol (random open coil) and gel (organized closed structure) for κ- and ι-carrageenan is not sharp, but is a progressive continuum from 100% sol to 100% gel, as either carrageenan concentration or gelling cations or both are increased. λ-Carrageenan is non-gelling at all concentrations and cation balances and is therefore always in the random open coil form in water. λ-Carrageenan normally occurs as a minor component in combination with κ-carrageenan in commercial products and enhances the gelling matrix through physical (void filling) and chemical (hydrogen-bonded helix cross-linkages) means. Carrageenan gels do not melt until temperatures are well above 37 °C.

The stability of carrageenan in foods is influenced by several factors, such as pH, direct structural bridging between the negatively charged carrageenan and positively charged protein sites, and indirect structural bridging with negatively charged protein sites via divalent cations such as calcium, through hydrogen bonding and through carrageenan–carrageenan helical interactions. Carrageenan is added to infant formula in order to stabilize the emulsion of protein, fat and water so as to maintain the consistency of the infant formula throughout storage and feeding.

Toxicological data

The form and stability of carrageenan in test materials and in foods are important considerations, both for interpretation of in vitro and in vivo experimental studies

on carrageenan and for assessment of the safety-in-use of carrageenan in a food product such as infant formula. Form and stability can be influenced by the preparation of the food and the nature of the matrix (solid or aqueous, presence or absence of protein) in which it is administered and by its passage through the gut. In previous studies, oral toxicity has been considerably influenced, not only by dose, but also by whether the test material administered was food-grade carrageenan or poligeenan. However, for some studies, the information on the specifications of the test material was inadequate, and sometimes no distinction was made between poligeenan and carrageenan in discussion and interpretation of results. Further, even if the type of carrageenan used is identified as commercial pure λ-, κ- or ι-carrageenan, analyses have shown that test materials may not be as described by the commercial supplier; the test materials may, for example, contain more than one type of carrageenan together with a substantial percentage of sucrose or dextrose.

Earlier studies on absorption in adult rats, guinea-pigs and primates have shown that little or no food-grade carrageenan is absorbed following oral exposure. At previous meetings, the Committee has commented that high molecular weight carrageenan is probably not absorbed. At the sixty-eighth meeting (Annex 1, reference *188*), it was noted that no studies were available addressing the effects of carrageenan on the immature gut, and it was not possible to draw conclusions on whether carrageenan might be absorbed from the immature gut.

For the present meeting, new data on absorption were available from good laboratory practice (GLP)–compliant studies in neonatal minipigs and pigs in which food-grade carrageenan was given in infant formula adapted for pig requirements. These studies used a liquid chromatography/tandem mass spectrometry analytical method that had been developed using poligeenan as a surrogate for detection of the low molecular weight tail of carrageenan in blood. In the minipig study, essentially no signal from low molecular weight carrageenan was detected in serum following oral administration of daily carrageenan doses of 0, 300 or 3000 mg/kg formula for 10 days starting on postnatal day 2, the higher dose being equal to 600–666 mg/kg bw per day. In the pig study, carrageenan doses of 0, 300, 1000 or 2250 mg/kg formula were given for 28 days starting on postnatal day 3, the highest dose being equal to 430–448 mg/kg bw per day. A positive signal corresponding to that expected for the low molecular weight tail of carrageenan was found in male piglets. However, the positive signal was observed in both treated and control males, irrespective of whether they had been given control formula or formula containing carrageenan. The range of values in control and carrageenan-treated males was similar and was not dose related. The signal was not seen in the female piglets, and it is likely that the signal corresponded to some other constituent of male serum, but this was not further investigated or identified.

The Committee concluded that absorption studies in neonatal minipigs and pigs are an appropriate model for the immature gut in human infants. However, because of the problems with the outcomes of the assay, the Committee was unable to conclude that fragments in the low molecular weight tail of carrageenan (molecular weight range 20–50 kDa) are not absorbed across the immature gut into the systemic circulation.

The suitability of carrageenan for use in infant formula requires additional considerations to those for the general use of carrageenan in foods. Of the information reviewed previously by the Committee, all animal experiments, apart from one in infant baboons given infant formula containing carrageenan, were performed in adult animals, which limits their usefulness for the safety evaluation of carrageenan for infants. From the absence of effects in the study in infant baboons, a no-observed-effect level (NOEL) of 1220 mg/L in formula was identified, equivalent to an exposure of 432 mg/kg bw per day. However, as the previous Committee commented at its sixty-eighth meeting (Annex 1, reference *188*), in this study, the colon was fixed in 10% buffered formalin, and this does not enable identification of mast cells that would be present if an inflammatory process had been initiated.

In the new GLP-compliant toxicological studies in neonatal minipigs and pigs that were submitted for this meeting, there was extensive microscopic study of sections taken from along the length of the intestine and the use of appropriate fixatives and stains for visualization of mucosal mast cells. Additionally, in the pig study, goblet cells in the villi and crypts of the jejunum were examined by appropriate staining. Immunophenotyping of leukocytes, measurement of proinflammatory cytokines interleukin-1 (IL-1), IL-6, IL-8 and tumour necrosis factor alpha (TNFα) in blood, and immunohistochemistry of the gut to assess the presence of IL-8 and TNFα were also carried out.

In the 10-day study in neonatal minipigs, no treatment-related effects were observed, apart from reduced feed consumption and reduced body weight gain on some days in male and female minipigs given the highest carrageenan concentration of 3000 mg/kg formula. Increased red blood cell count, haemoglobin and haematocrit were also seen in males given a carrageenan concentration of 3000 mg/kg formula, which may have been related to the reduced consumption of formula in the high-dose group. Detailed microscopic assessment of the gastrointestinal tract showed no evidence of inflammation or other lesions, and there was no effect of carrageenan treatment on mucosal mast cell counts. The NOAEL for carrageenan from this study was 300 mg/kg formula, equal to an exposure of 74 mg/kg bw per day, but it should be noted that the effects seen at the higher dose were likely attributable to reduced palatability and the reduced feed consumption at that dose.

In the 28-day neonatal pig study, in which the highest carrageenan concentration of 2250 mg/kg formula was less than the highest concentration in the minipig study, there were no treatment-related effects on body weight or feed consumption. There were also no treatment-related effects on haematology, clinical chemistry, organ weights, or organ and tissue histopathology. The extensive microscopic assessment of the gastrointestinal tract showed no evidence of inflammation or other lesions, and there was no effect of carrageenan treatment on mucosal mast cell counts. Similarly, there were no treatment-related effects on immunophenotyping of blood lymphoid subsets or proinflammatory cytokines in the blood or in the gut. The NOAEL for carrageenan from this study was 2250 mg/kg formula, equal to an exposure of 430 mg/kg bw per day.

The Committee considered that the neonatal pig and minipig studies in which formula containing carrageenan was given during the first month of life were appropriate to model the human infant from 0 to 12 weeks of age, when infant formula may be provided as the sole source of nutrition. It is also a relevant model for the decrease in permeability of the gut epithelium to macromolecules during the neonatal period ("gut closure") and immunological development. The type of carrageenan administered (predominantly κ-carrageenan) and the use of adapted infant formula as the matrix for the studies also adequately reflected the types of infant formula containing carrageenan available for human infants. The Committee noted that although these are liquid rather than solid foods, infant formula contains proteins that bind carrageenan. The pig and minipig studies have provided considerable information to address the issues of whether inflammation occurred in neonatal animals and whether any damage was done to the gut mucosa. No adverse effects were observed on the gut or on the immune parameters assessed. The Committee concluded that the NOAEL from the neonatal pig study could be used to estimate MOEs for human infants consuming formula containing carrageenan.

The recent in vitro studies on inflammatory pathways reviewed at this meeting and the results of some of the studies reviewed by the Committee at previous evaluations raise the question of whether carrageenan might play a role in intestinal inflammation. The Committee agreed with the problems that have been pointed out by others in some of the methodological aspects of the in vitro studies reviewed at this meeting. In addition, the Committee noted that the in vitro studies with carrageenan were not validated by assessment of responses to a positive control, such as a known inflammatory substance. The Committee also had concerns about the use of proliferating cell lines, as inflammatory effects in the gut would be expected to be exerted on the mucin-producing cells at the tips of the intestinal villi and not on the proliferating cells in the crypts. More importantly, the Committee considered that there are also difficulties in extrapolating findings from in vitro studies on human intestinal cell cultures to draw conclusions on risk

assessment for humans in vivo. This aspect is particularly relevant given that in vitro systems reflect only one component of the in vivo processes for prevention of gut inflammation, which are known to be complex.

It is also evident that the observations on activation of inflammatory or mitogenic signalling pathways in human colonic cells cultured in vitro are in contrast to findings from in vivo studies in which carrageenan has been given in the diet. There is no evidence of intestinal inflammation or lesions in laboratory animal studies in which well defined, undegraded carrageenan, which nevertheless has a small proportion of lower molecular weight components, has been given orally in the diet, in contrast to some results from administration in the drinking-water. There may be several reasons for this, including that in vivo the cells lining the gastrointestinal tract are protected by a mucous barrier that is not present in in vitro models and that dietary administration, as opposed to drinking-water administration, offers the opportunity for carrageenan to bind to protein.

Human studies

The Committee noted that only two brief reports of studies on infants given formula containing carrageenan are available. Both were conducted in healthy newborn infants. In one 6-month study, 1269 infants were given infant formula containing carrageenan at 300 mg/L and compared with 149 infants given formula with no carrageenan. In the other 112-day study, 100 infants were fed formula containing carrageenan at 1000 mg/L and compared with 95 infants given formula with no carrageenan; 58 infants failed to complete the trial, but the numbers dropping out due to intolerance were similar in both groups. Detailed descriptions of these studies were not available, and the brief reports did not indicate any health problems in either study.

Assessment of dietary exposure

The typical level of carrageenan used in reconstituted powdered and liquid cow milk– and soy-based formulas is 0.009–0.1 g/100 mL (90–1000 mg/L), with the higher levels being used in formulas containing hydrolysed proteins. Current use levels include 0.03 g/100 mL (300 mg/L) for regular milk- and soy-based liquid formulas and 0.1 g/100 mL (1000 mg/L) for hydrolysed protein– and/or amino acid–based liquid formulas.

Median infant formula consumption estimates were derived from estimated energy requirements for fully formula-fed infants. It should be noted that the energy requirements of formula-fed infants are greater than those of breastfed infants, although this disparity decreases with increasing age. A further exposure scenario was considered, using high (95th percentile) daily energy

intakes reported for formula-fed infants. The highest reported 95th percentile energy intakes were for infants aged 14–27 days. For all dietary exposure estimates, a common energy density of formula of 67 kcal/100 mL (280 kJ/100 mL) was used to convert energy to the volume of formula ingested daily.

For infants aged 0–6 months, median dietary exposure to carrageenan was estimated to be in the range 37–54 mg/kg bw per day for carrageenan added to infant formula at 300 mg/L formula, as consumed. At a use level for carrageenan of 1000 mg/L formula, as consumed, median estimated dietary exposure was in the range 124–180 mg/kg bw per day. High (95th percentile) exposures to carrageenan were estimated to be 65–67 mg/kg bw per day for a use level of 300 mg/L and 218–222 mg/kg bw per day for a use level of 1000 mg/L.

Evaluation

New studies relevant to the evaluation of the use of carrageenan in infant formula and formula for special medical purposes have been conducted since the Committee last considered this issue (Annex 1, references *187* and *188*). They include investigations of absorption and toxicity in both the neonatal minipig and neonatal pig in which carrageenan was fed in infant formula adapted for piglets.

The absorption studies did not allow any conclusions to be reached. In the toxicity studies, in addition to a wide range of toxicological parameters, a detailed examination of the histology of all segments of the gastrointestinal tract and quantification of mast cells along the gastrointestinal tract were undertaken in both the minipig and pig. In the pig, an appropriate array of serum and gut cytokines was also assessed, together with blood leukocyte immunophenotyping. From these new investigations, there was no evidence of any inflammation in the gut or any effects on immune parameters. A NOAEL of 430 mg/kg bw per day, which was the highest dose tested, was derived from the neonatal pig study. The Committee also noted that the NOAEL of 430 mg/kg bw per day from the neonatal pig study is almost identical to that from the earlier infant baboon study of 432 mg/kg bw per day.

In the 10-day neonatal minipig study, animals were given infant formula containing carrageenan at concentrations up to 3000 mg/kg (0.3%). Concentrations of carrageenan above approximately 2500 mg/kg (0.25%) become highly viscous, and this appears to have adversely affected palatability and growth in the minipigs. Accordingly, the amount of carrageenan added to the formula fed to piglets in the main study was reduced to 2250 mg/kg (0.225%). As a consequence of this limitation, the MOEs between the NOAEL from the pig study and human infant exposures at 2–4 weeks of age range from 2 to 12 on a body weight basis and from 2 to 8 on a concentration basis.

The Committee noted that although the MOEs are small in magnitude (see section 2.5), they are derived from a neonatal pig study in which the highest dose tested was without adverse effects on the gut or on immune parameters, supported by a neonatal minipig study. The neonatal pig and minipig are appropriate models for the young human infant up to at least 12 weeks of age, for whom infant formula may be the sole source of nutrition. These new studies allay the earlier concerns that carrageenan, which is unlikely to be absorbed, may have a direct effect on the immature gut. The Committee also took account of the previous toxicological database on carrageenan, which did not indicate other toxicological concerns.

The Committee concluded that the use of carrageenan in infant formula or formula for special medical purposes at concentrations up to 1000 mg/L is not of concern. The Committee recognizes that there is variability in medical conditions among infants requiring formulas for special medical purposes that contain the higher levels of carrageenan, and the Committee notes that these infants would normally be under medical supervision.

The Committee at its sixth-eighth meeting (2007) had prepared specifications for carrageenan. The Committee discussed limits on lead specifications for this and the other food additives for use in infant formulas that were on the agenda, as described in section 2.4.1. The Committee revised the specifications with minor changes.

An addendum to the toxicological monograph was prepared.

3.1.3 Citric and fatty acid esters of glycerol (CITREM)

Explanation

Citric and fatty acid esters of glycerol (CITREM) are listed in the GSFA *(3)* for use as an antioxidant, emulsifier, flour treatment agent, sequestrant and stabilizer in several food categories.

The Committee previously reviewed CITREM at its seventeenth (1973), thirty-fifth (1989) and sixty-first (2003) meetings (Annex 1, references *32*, *88* and *166*). At its seventeenth meeting, the Committee allocated an ADI "not specified" to CITREM. The Committee based its safety evaluation on biochemical and metabolic studies demonstrating that this substance is completely hydrolysed in the gastrointestinal tract into components that are normal constituents of the diet, together with knowledge of the metabolism and lack of toxicity of citric acid, glycerol and fatty acid esters of glycerol.

At the request of CCFA at its Forty-fifth Session *(9)*, the Committee evaluated the safety of CITREM for use as an emulsifier in infant formula and formula for special medical purposes intended for infants, to replace the combined use of three emulsifiers – lecithin, monoglycerides and diglycerides

of fatty acids, and diacetyl tartaric acid ester of monoglycerides and diglycerides. The proposed use levels considered at this meeting were up to 7.5 g/L as consumed in reconstituted infant formula powder and up to 9 g/L in ready-to-feed liquid infant formula. The higher amounts are used in formulas based on amino acids or (partially) hydrolysed protein.

Chemical and technical considerations

CITREM (INS 472c) is a white to ivory coloured, oily to waxy material. It is a mixture of citric acid esters and fatty acid esters of glycerol and is obtained by esterification of glycerol with citric acid and food-grade fatty acids or by reaction of a mixture of monoglycerides and diglycerides of food-grade fatty acid with citric acid. The structural formula for CITREM is shown below:

$$\begin{array}{l} CH_2-OR_1 \\ | \\ CH-OR_2 \\ | \\ CH_2-OR_3 \end{array}$$

where at least one of R_1, R_2 or R_3 represents a citric acid moiety or a fatty acid moiety and the remainder may represent citric acid, fatty acid or hydrogen. CITREM is mainly composed of glycerol (8–33%), fatty acids (37–81%) and citric acid (13–50%) and could contain up to 4% of free glycerol, minor amounts of free fatty acids, free citric acid, and monoglycerides and diglycerides. CITREM may be wholly or partially neutralized with sodium hydroxide or potassium hydroxide.

Biochemical data

A newly available in vitro study on the digestibility of CITREM itself and CITREM-containing infant formula confirms that hydrolysis of CITREM by gastric and pancreatic lipases occurs under conditions in which the pH and amount of bile salts are varied to mimic those in the stomach and duodenum of term and preterm human infants. However, in this two-stage in vitro model, when CITREM in infant formula was added, hydrolysis of CITREM into its component parts of glycerol, citric acid and fatty acids was incomplete and in the range of 14–28%; this was lower than expected. The likely reason for the partial hydrolysis was shown in further studies in the two-stage in vitro model in which hydrolysis of pure citric acid and fatty acid esters of glycerol (i.e. the main components of CITREM without any free glycerol, free citric acid, free fatty acids or free glycerides) was found to be around 2-fold higher (47–58%) than for CITREM in infant formula. This confirmed the suspected negative effect of free glycerides on the action of lipases. These data suggest that, in vivo, hydrolysis of

CITREM is likely to continue lower down the small intestine as the glycerides and free fatty acids from breakdown of the fats in infant formula and CITREM form micelles and are progressively absorbed by the enterocytes. Absorption of the contents of the micelles occurs mainly in the proximal jejunum and partly in more distal segments of the small intestine, which are not modelled in the two-stage in vitro model. The model also did not include lingual lipase, which would contribute to overall hydrolysis in vivo.

Toxicological data

There are few toxicological studies available on CITREM from previous evaluations. The only new information available on CITREM for this evaluation is a report that an in vitro study on *Salmonella typhimurium* did not show any evidence of gene mutations and a short-term study on the effects of CITREM on fat absorption in the rat, which was not considered useful for the evaluation because of the very high amount of CITREM used.

The Committee considered the hypothesis, based mainly on in vitro studies, that food emulsifiers may decrease the integrity of the intestinal epithelial barrier. The evidence for such a hypothesis is limited, particularly because in the in vitro studies, surfactants and emulsifiers have been applied directly to cells at concentrations (e.g. 1 mg/mL) that are likely to exceed those occurring normally in the gut lumen following consumption of foods containing emulsifiers. It should also be noted that monolayers of a human colon cancer–derived cell line (CaCo2) used in these studies do not mimic physiological conditions; for example, they lack goblet cells that secrete mucin, so they do not have the protective layer of intestinal mucus that would be present in vivo. None of the in vitro studies used CITREM. Several studies using emulsifiers with significantly higher hydrophilic–lipophilic balance (HLB) values than those of CITREM showed disruption of epithelial integrity. Although the surfactant activity of food emulsifiers demonstrated in in vitro models makes it relevant to consider this hypothesis, it is necessary to take account of the differences between normal physiological conditions in the gut during food digestion, including the amounts of emulsifier present, the hydrolysis and dilution of the emulsifier, and the conditions of the experimental studies, before concluding on relevance for health. It is not possible to conclude from the studies conducted to date on emulsifiers other than CITREM, with higher HLB values than those of CITREM, that CITREM itself will affect the intestinal barrier under in vivo conditions.

Human studies

Tolerance of infants to formulas containing CITREM at concentrations ranging from 0.95 to 1.62 g/L is supported by a number of clinical studies. Similarly, a

formula containing added citric acid salt at 2.5 g/L was also well tolerated by infants. No clinical trials on tolerance of infants to formulas containing higher amounts of CITREM, up to the maximum of 9 g/L used in some formulas for special medical purposes intended for infants, have been submitted to the Committee. The Committee was provided with a summary of five case reports on infants aged 2–11 months given a liquid, peptide-based formula containing a high concentration of CITREM (8.56 g/L) for 2 or 4 weeks. These did not allow any conclusions to be drawn on tolerance, as all the infants had pre-existing gastrointestinal disorders or diseases; some additionally had other health problems; and some had loose or soft stools before starting on the formula.

The Committee considered whether there may be adverse effects from citric acid released in the gut from CITREM. Citric acid has been evaluated previously by the Committee (Annex 1, reference *33*) and given an ADI "not limited",[3] but this evaluation did not cover infants less than 12 weeks of age. The estimated total exposure of infants in the age range 14–27 days (the age range with the highest exposures) to citric acid, derived from both the natural constituents of formula and CITREM added to formula at 9 g/L, is up to 930 mg/kg bw per day for infants with median energy requirements and up to 1140 mg/kg bw per day for infants at the 95th percentile energy intake, if citric acid is assumed to be present at the upper end of the range reported for CITREM (13–50%).

Oral rehydration solutions delivering citrate at about 98 mg/kg bw per day were without adverse effects in a double-blind randomized control study in infants and children. A study in low birth weight infants, preterm infants and term infants on the effect of supplementation of cow's milk formula with citrate salts, giving doses of about 500 mg/kg bw per day, for 3 weeks from birth reported no adverse effects on growth or tolerance, including stool frequency, compared with controls not receiving citrate. However, in a small study in which free citric acid was given in divided doses over 24 hours, diarrhoea occurred in four of eight infants aged 4–12 months receiving total amounts (food citrate plus free citric acid) equivalent to a citric acid exposure of approximately 400–700 mg/kg bw per day. The Committee noted that citric acid–based oral rehydration solutions in which trisodium citrate (2.94 g/L) or tripotassium citrate (3.24 g/L) is used to replace bicarbonate have been successfully used in the treatment of diarrhoea in infants and adults. Oral sodium or potassium citrate, taken as tablets or in solution in divided doses, is also prescribed to alkalinize the urine for the treatment of urinary tract infections, hypocitraturia and kidney stones, including at doses of 1–4 mEq/kg bw per day (108–430 mg/kg bw per day) in infants and children. Diarrhoea is listed as an occasional side-effect of potassium citrate treatment, due to the irritant effect in the gut. It is also noted that formula of a similar composition to some formulas for

[3] A term no longer used by JECFA that has the same meaning as ADI "not specified".

special medical purposes intended for infants, but not containing CITREM, has been associated with softening of stools in infants.

Assessment of dietary exposure

Dietary exposures were estimated for a typical use level of CITREM of 2.7 g/L in powdered infant formula after reconstitution and the upper range of requested use levels of CITREM at 7.5 g/L and 9 g/L, for use in formula for special medical purposes intended for infants. For CITREM subcomponents, it was assumed that complete hydrolysis into its free components would occur. Of the components of CITREM, only citric acid was identified as requiring an exposure estimate from consumption of formula containing CITREM. It has been reported that CITREM may contain 13–50% citric acid. Basal levels of citric acid in "typical" prepared infant formulas (0.64 g/L) were also considered in the assessment of exposure to citric acid.

Median infant formula consumption estimates were derived from estimated energy requirements for fully formula-fed infants. It should be noted that the energy requirements of formula-fed infants are greater than those of breastfed infants, although this disparity decreases with increasing age. A further exposure scenario was considered, using high (95th percentile) daily energy intakes reported for formula-fed infants. The highest reported 95th percentile energy intakes were for infants aged 14–27 days. For all dietary exposure estimates, a common energy density of formula of 67 kcal/100 mL (280 kJ/100 mL) was used to convert energy to the volume of formula ingested daily.

For infants aged 0–6 months and a 2.7 g/L CITREM use level, median estimated exposures to citric acid are in the range 120–360 mg/kg bw per day, with the high end of the range relating to infants 0–1 month of age and a citric acid content for CITREM of 50%. At the high end of intended use (9 g/L), the median citric acid exposure estimates are in the range 230–930 mg/kg bw per day. For 95th percentile consumers 14–27 days of age and considering a 2.7 g/L CITREM usage, estimated citric acid exposures are up to 440 mg/kg bw per day; at the higher requested use level (9 g/L), the 95th percentile citric acid exposure estimates are up to 1140 mg/kg bw per day for a citric acid content for CITREM of 50%.

Assuming that CITREM would have an energy density equivalent to that of triglycerides (9 kcal/g), it has been estimated that CITREM is a low contributor to energy intakes (3.6–12.1%), depending on the use level.

Evaluation

The new study on CITREM digestibility in a two-stage in vitro model mimicking the stomach and duodenum of preterm and term infants showed incomplete hydrolysis of CITREM. However, the model did not simulate the entire digestion

process or the further hydrolysis that is likely to occur lower down the gut. The Committee concluded that CITREM was likely to be substantially hydrolysed in the gut in vivo and that any partially hydrolysed products, such as glycerol citric acid esters, would not be of safety concern.

The Committee considered the limited available evidence on whether free citrate in the gut would cause diarrhoea. In one study, no effects were observed in 13 infants exposed to free citrate, given as citrate salts added to formula, at 500 mg/kg bw per day. In another study, diarrhoea was observed in four out of eight infants given free citric acid by gavage in divided doses over 24 hours, equivalent to a total exposure to free citrate of approximately 400–700 mg/kg bw per day. The Committee noted that the diarrhoea may have been due to osmolality and the gavage mode of administration. In the gut, the enzymatic release of free citrate from infant formula containing CITREM would be more gradual. The Committee was also aware that citrate salts have been used in oral rehydration solutions for the treatment of diarrhoea in infants. Clinical trials in infants show tolerance to formulas containing CITREM at up to 1.6 g/L, but there are no tolerance trials in infants given formula containing CITREM at the high end of the requested range (9 g/L).

Taking the above considerations into account, it is unlikely that consumption of formulas containing typical levels of CITREM used in powdered formulas (up to 2.7 g/L as reconstituted), which is equivalent to an exposure to citrate of 440 mg/kg bw per day for the very young infant at the 95th percentile energy intake, would cause diarrhoea. At the high end of the requested range (up to 9 g/L), which is equivalent to an exposure to citrate of 1140 mg/kg bw per day for the very young infant at the 95th percentile energy intake, diarrhoea might occur in some infants.

The Committee concluded that there are no toxicological concerns about the use of CITREM in infant formula and formula for special medical purposes at concentrations up to 9 g/L. At the higher use levels, there is a possibility of diarrhoea from free citric acid released from formula containing CITREM. Given the paucity of clinical data and the fact that exposure assumptions for citric acid have been maximized, it is difficult to estimate the risk of diarrhoea, but it is considered to be low.

At the present meeting, the Committee reviewed the specifications for CITREM. The Committee discussed limits on lead specifications for this and the other food additives for use in infant formulas that were on the agenda, as described in section 2.4.1. The Committee also noted that the test method for the determination of total citric acid currently employs a gas chromatographic method using a packed column. The Committee recommends replacing this method with a suitable method using a capillary/wide-bore column, for consideration at a future meeting.

Based on the information available, the Committee revised the existing specifications, making minor changes to the purity tests for CITREM.

An addendum to the toxicological monograph was prepared.

3.1.4 Gardenia yellow

Explanation

Gardenia yellow is an extract from the fruit of *Gardenia jasminoides* Ellis that is used as a food colour in some countries. The main colouring principals of gardenia yellow are crocetin and crocin. Crocetin and crocin also occur in saffron, which was evaluated by the Committee at its fourth, twenty-first and twenty-ninth meetings (Annex 1, references *4*, *44* and *70*). Saffron was regarded as a food rather than a food additive.

Gardenia yellow has not been evaluated previously by the Committee. It was on the agenda at the request of the Forty-fifth Session of CCFA *(9)*.

Chemical and technical considerations

Gardenia yellow is produced by ethanol extraction from the fruits of *Gardenia jasminoides* Ellis and subsequent purification. The colouring principals of gardenia yellow are the carotenoid crocetin and crocetin esters. Crocin (crocetin di-gentiobiose ester) is the major crocetin ester. Geniposide, a substance with reported therapeutic effects, may also be present in the final product at up to 0.5%.

The Committee was unable to evaluate the chemical characteristics of the product owing to the lack of relevant data and because of inconsistent and contradictory information provided by the two sponsors.

Biochemical data

The available data show that when given orally as a single dose, crocin, the main component of gardenia yellow, is not absorbed. Deglycosylation of crocin in the intestinal tract produces crocetin, which is then rapidly absorbed and distributed. It is claimed in the submission that crocetin is absorbed following the same pathway as for other carotenoids (e.g. β-carotene, lutein, lycopene). Evidence was provided that absorbed crocetin is partly metabolized to monoglucuronide and diglucuronide conjugates in mice and has an elimination half-life of about 7 hours in humans.

Toxicological data

No acute toxicity was reported in studies with mice and rats at doses of gardenia extract (composition unknown) up to 20 g/kg bw and 4 g/kg bw, respectively. Mice received a single intragastric administration of an aqueous solution of gardenia yellow powder at a dose of 10, 15 or 20 g/kg bw. This powder was

described by the authors as containing 92% of a gardenia extract with crocin as the main component (the method of preparation was not given). Rats received a single intragastric administration of either a suspension in water of the same preparation of gardenia yellow powder (females: 3 g/kg bw; males: 4 g/kg bw) or a water extract of gardenia yellow of unknown preparation and composition (3 g/kg bw for both males and females).

In a 13-week study, rats were fed gardenia yellow extract at a concentration of 0, 750, 1500 or 3000 mg/kg diet (equivalent to 0, 75, 150 and 300 mg/kg bw). Gardenia yellow intakes were calculated by the authors as 0, 35, 72 and 143 mg/kg bw per day for males and 0, 43, 88 and 166 mg/kg bw per day for females, respectively. The gardenia yellow powder added to the diet was described as containing 92% of a gardenia extract, with crocin being stated as the main component. A NOAEL for gardenia yellow of 72 mg/kg bw per day, corresponding to 1500 mg/kg in the diet, was identified by the authors, based on increased serum aspartate transaminase activity together with an accumulation of lipid droplets in the hepatocytes seen at 3000 mg/kg diet, the highest concentration tested. The Committee noted that the exact composition of the material tested was not described, the weights of the animals at the end of the study and the feed consumption were not provided, and details on the observed effects were not reported.

In another 13-week study, no effects were reported in rats with dietary exposure to gardenia yellow equivalent to 300 mg/kg bw per day. The Committee noted that the composition of the gardenia yellow used was not known.

No long-term toxicity or carcinogenicity studies were available.

Gardenia yellow (composition unknown) was tested in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 using the plate incorporation and the preincubation methods. In the preincubation test, the concentrations of gardenia yellow were 10, 25 and 50 mg/mL. No genotoxicity was reported. Another Ames test with concentrations of gardenia yellow (composition not given) up to 50 mg/plate was also negative, with and without metabolic activation.

In V79 cells, gardenia yellow (15.6–1000 μg/mL of a water-soluble extract from *Gardenia jasminoides*) caused DNA damage in the rec-assay at all the concentrations tested and induced a significant dose-dependent increase in sister chromatid exchange frequency. Three-dimensional capillary electrophoresis analysis of the extract did not show any genipin (a substance with known genotoxic potential formed by hydrolysis of geniposide), and there was only one peak, which was considered by the authors to correspond to geniposide. Geniposide did not show any genotoxic activity in this study. The Committee noted that the compound responsible for the genotoxic effect was not identified.

In an in vivo bone marrow micronucleus test, mice received a single dose of gardenia yellow (composition unknown) in aqueous solution by gavage at 2.5, 5 or 10 g/kg bw. No genotoxic effects were seen.

No multigeneration reproductive toxicity studies were available.

Mice receiving an aqueous solution of gardenia yellow (composition unknown) by gavage for 5 consecutive days at a dose of 2.5, 5 or 10 g/kg bw per day were terminated 35 days after the first day of treatment. No morphological abnormalities were reported in the sperm of the mice of the lower two dose groups. All animals that received 10 g/kg bw per day for 5 days died before the end of the study.

No developmental toxicity studies were available, with the exception of a study using *Xenopus* embryos. A teratogenic potential for crocetin was reported. The Committee considered this study to be not relevant for its evaluation of gardenia yellow.

Several special studies were presented in the submission. The Committee noted that most of these studies were not designed to evaluate potential adverse effects. In addition, the studies used saffron as the source of crocin, and most of them described therapeutic effects of purified crocin. Therefore, the Committee considered these studies to be not relevant for its safety evaluation of gardenia yellow.

Two clinical trials in humans were reported. In a double-blind, placebo-controlled, three-way crossover study designed to examine the effects of crocetin on physical fatigue compared with placebo, 14 healthy volunteers (seven men and seven women) were randomized to daily oral administration of capsules containing 15 mg crocetin or placebo for 8 days. The Committee noted that the study was not designed to evaluate adverse effects.

In order to investigate the effect of crocetin (extracted from gardenia yellow; chemical analysis not presented) on sleep, a clinical trial was undertaken comprising a double-blind, placebo-controlled crossover trial of 21 healthy adult men with a mild sleep complaint. Crocetin was administered at 7.5 mg/day in a gelatine capsule during two intervention periods of 2 weeks separated by a 2-week washout period. Body weight, blood pressure and pulse rate, as well as haematological and biochemical analysis of blood samples, did not show significant differences after intake of crocetin compared with placebo.

The Committee noted that the usefulness of these clinical trials was limited for the evaluation of gardenia yellow, as they were of short duration and the administered doses were low.

No epidemiological studies were reported.

Assessment of dietary exposure

Owing to the uncertainties in the composition of the gardenia yellow used, the Committee did not perform an exposure assessment. Uses and use levels in foods are proposed in the submission, and the resulting daily exposures were calculated by the sponsor based on national food consumption data for the Chinese population. Average exposure estimates ranged from 5.5 to 18 mg/kg bw per day for the populations of "females over 18 years" and "1–3 years", respectively. At the 95th percentile, the dietary exposures ranged from 22.8 to 76.9 mg/kg bw per day for these populations.

Evaluation

The Committee noted that the manufacturing process and composition of gardenia yellow were insufficiently described.

The Committee also noted that some studies included in the submission were performed using extracts from *Crocus sativus*, and the possible similarities and differences with the food colour prepared from *Gardenia jasminoides* were not documented. In many of the available studies, the material tested was not well characterized and/or not adequately described. Therefore, it is not clear whether the material tested toxicologically was representative of gardenia yellow.

The Committee further noted that the available toxicity studies have not been conducted following internationally recognized guidelines and that a number of studies were performed using non-relevant (intraperitoneal or intravenous) routes of administration. There are no long-term toxicity, carcinogenicity, reproductive toxicity or developmental toxicity studies available.

Because of the inconsistent and contradictory data provided by the two sponsors, the Committee was unable to prepare a specifications monograph.

In order to establish specifications, the Committee requires:

- information on the manufacturing process, including purification steps;
- analytical data on the composition of the substance, including the total amount of colouring matter and relevant compounds of known biological activity, such as geniposide and genipin;
- data on loss on drying;
- information on a method of assay;
- analytical data on at least five different batches of commercial materials supporting the specifications; and
- data on stability in food.

Given the deficiencies in the toxicological database, including missing toxicological studies (e.g. long-term toxicity, carcinogenicity, reproductive toxicity and developmental toxicity studies), the inadequate characterization of the test material and limited reporting of the available studies, the Committee was unable to evaluate gardenia yellow proposed for use as a food colour.

A toxicological monograph was prepared.

3.1.5 Lutein esters from *Tagetes erecta*

Explanation

Lutein esters contain lutein, (all-*E*,3*R*,3′*R*,6′*R*)-β,ε-carotene-3,3′-diol, a naturally occurring xanthophyll pigment. Lutein occurs with its isomeric xanthophyll zeaxanthin in many foods, particularly vegetables and fruit. Extracts containing xanthophylls (free and/or esterified) are used as colours and as nutritional supplements in a wide range of applications.

Products extracted from *Tagetes erecta* containing lutein and its esters have been the subject of previous JECFA evaluations. At its thirty-first meeting (Annex 1, reference *77*), the Committee prepared tentative specifications for xanthophylls obtained from *Tagetes erecta* petals, but no toxicological data were available, and no toxicological evaluation was performed. *Tagetes* extract containing lutein esters at low concentrations was considered by the Committee at its fifty-fifth and fifty-seventh meetings (Annex 1, references *149* and *154*), and the tentative specifications were revised (Annex 1, reference *151*) and then superseded by full specifications (Annex 1, reference *156*). At its sixty-third meeting (Annex 1, reference *173*), the Committee evaluated biochemical data and the results of toxicological and human studies on *Tagetes* preparations with a high content of unesterified lutein (>80%) and established a group ADI of 0–2 mg/kg bw for lutein from *Tagetes erecta* and synthetic zeaxanthin.

At the present meeting, *Tagetes* extract was placed on the agenda following a request by CCFA to undertake a safety assessment and revision of specifications. However, the Committee noted that the information supplied by the sponsor referred to a substance with a higher content of carotenoid esters (>60%) compared with *Tagetes* extract; therefore, the Committee decided to name this extract "Lutein esters from *Tagetes erecta*". The INS number assigned to *Tagetes* extract could not be used as a synonym for this product.

Chemical and technical considerations

Lutein esters from *Tagetes erecta* is a dark yellow-brown solid insoluble in water and soluble in hexane. The product is obtained by solvent extraction of dried petals of *Tagetes erecta* L. (marigold), further purification and subsequent removal of solvents. Lutein esters account for the major part of the extract, and a smaller

proportion of zeaxanthin esters is also present, together with other carotenoids (xanthophylls), either free or as monoesters or diesters of fatty acids. The esters contain saturated long-chain fatty acids, such as myristic, palmitic and stearic acid, in various proportions, with palmitic acid being a major component. Waxes and fatty acid–containing moieties naturally occurring in the source material may also be present. Lutein, (all-*E*,3*R*,3′*R*,6′*R*)-β,ε-carotene-3,3′-diol, is an oxygenated carotenoid often occurring with its isomeric xanthophyll zeaxanthin.

Biochemical data

The results of a number of studies conducted in experimental animals and humans have shown that the administration of lutein esters – in particular, lutein esters from *Tagetes erecta* – as dietary constituents or as nutritional supplements can lead to increases in levels of lutein in blood and accumulation of lutein in tissues (liver, adipose, eye). Lutein is also present in human milk. As assessed by increases in plasma levels of lutein, the bioavailability of lutein from the administration of lutein esters is equivalent to that from molar equivalent doses of lutein. Based on data obtained in studies with lutein, which generally report bioavailability in the range of 5–11%, the overall bioavailability of lutein from lutein esters is expected to be low, but may be higher with high fat intake.

Toxicological data

The toxicological study considered by the sixty-third meeting of the Committee (Annex 1, reference *173*) for the establishment of the ADI for lutein was a 13-week study in rats, from which the NOAEL was 200 mg/kg bw per day, the highest dose tested. In addition, results of in vitro and in vivo genotoxicity tests were negative, as were the results of a developmental toxicity study in rats at doses up to 1000 mg/kg bw per day, the highest dose tested.

The present Committee noted that the acute toxicity of lutein esters from *Tagetes erecta* in rats is low. No evidence of toxicity was apparent within 14 days of a single oral dose of lutein esters at 3750 mg/kg bw (equivalent to 2025 mg/kg bw expressed as lutein equivalents) or within 12 days following a single administration of a lutein ester preparation that provided up to 4000 mg/kg bw lutein equivalents.

In repeated-dose studies up to 90 days, no evidence of adverse effects was observed in rats at lutein ester doses up to 1000 mg/kg bw per day (equivalent to 540 mg/kg bw per day expressed as lutein equivalents), the highest dose tested. Long-term studies of toxicity in laboratory animals were not available.

The genotoxic potential of lutein esters from *Tagetes erecta* was evaluated in a bacterial reverse mutation assay, an in vitro mouse lymphoma assay and an

in vivo rat bone marrow micronucleus test. Lutein esters from *Tagetes erecta* were not mutagenic or genotoxic in any of these tests.

Reproductive toxicity studies in laboratory animals were unavailable. In a study of developmental toxicity with lutein esters in rats, there was no evidence of maternal or fetal toxicity at doses up to 540 mg/kg bw per day expressed as lutein equivalents (1000 mg/kg bw per day expressed as lutein esters), the highest dose tested.

Human studies

The available data evaluated by the Committee at its sixty-third meeting (Annex 1, reference *173*) indicated that dietary lutein is well tolerated in humans. After 20 weeks of mixed lutein ester supplementation at 15 mg/day, the effects in humans were limited to a reversible yellowish skin discoloration in some subjects.

The present Committee evaluated a number of studies in humans that have investigated the effects of the administration of lutein esters, primarily as nutritional supplements, on plasma levels of lutein, on the level of the xanthophyll-containing macular pigment and as a therapeutic agent for age-related macular degeneration. Although these studies in humans were not designed as part of the safety assessment process, it is noted that no adverse effects were reported in these published studies with doses of lutein esters from *Tagetes erecta* up to 30 mg/day for up to 140 days.

A clinical trial in preterm infants (<33 weeks of gestation) to assess potential retinal effects of daily lutein and zeaxanthin supplementation (0.14 mg + 0.0006 mg, respectively) for 36 weeks did not reveal any adverse effects.

Assessment of dietary exposure

Lutein esters from *Tagetes erecta* would be used in the same food categories and at the same use levels as those evaluated for lutein from *Tagetes erecta* at the sixty-third meeting of the Committee (Annex 1, reference *173*). That Committee estimated mean and 90th percentile dietary exposures for lutein plus zeaxanthin of approximately 7 and 13 mg/day, respectively (equivalent to 0.12 and 0.22 mg/kg bw per day, assuming a 60 kg body weight). Based on these results and assuming a conversion factor of 1.8 to reflect the increased molecular weight due to the fatty acid moieties, the present Committee estimated that exposure to lutein esters from *Tagetes erecta* would be up to 24 mg/day (equivalent to 0.4 mg/kg bw per day, assuming a 60 kg body weight). The use of lutein esters from *Tagetes erecta* is considered to be substitutional for the use of lutein from *Tagetes erecta* and therefore would not increase the dietary exposure to lutein.

At the sixty-third meeting, the Committee reported estimates for the dietary exposure to lutein from natural sources to be in the range of 1–6 mg/

day (approximately 0.01–0.1 mg/kg bw per day) based on a number of studies in North America and the United Kingdom. EFSA estimated that the mean dietary exposure to lutein from the diet due to its natural occurrence is up to 0.1 and 0.04 mg/kg bw per day for children and adults, respectively.

Evaluation

As the lutein (and zeaxanthin) esters from *Tagetes erecta* considered in the present evaluation undergo hydrolysis to lutein or zeaxanthin prior to systemic absorption, the biochemical and toxicological data on non-esterified lutein and zeaxanthin are relevant to the safety assessment of the lutein esters.

At the present meeting, the Committee concluded that there were sufficient toxicological data to complete a safety assessment of lutein esters from *Tagetes erecta*. The Committee considered the available toxicological data for lutein and lutein esters, together with the dietary exposure of the general population to lutein and lutein esters. New 13-week studies in rats with lutein esters resulted in a NOAEL of up to 540 mg/kg bw per day expressed as lutein equivalents, the highest dose tested. Additional information to support the safety assessment of lutein esters from *Tagetes erecta* includes the absence of any adverse effects in genotoxicity and developmental toxicity studies; and the absence of any reported adverse effects in humans administered lutein esters. These new data support the findings for other lutein preparations considered by previous Committees. No reproductive toxicity studies were available, but the Committee noted that the material is a common component of the diet, with no toxicity reported in available studies.

The Committee also noted that the human fetus is exposed to varying concentrations of lutein and zeaxanthin in utero, depending on maternal dietary exposure. Newborn infants continue to bioaccumulate lutein in many tissues, such as the retina, as a consequence of lutein being present in human milk.

The Committee concluded that there was no need to establish a numerical ADI. This decision was based on a number of factors, including the absence of any observed toxicity of lutein or lutein esters in any of the available toxicological studies in animals; the absence of any adverse effects in humans consuming lutein or lutein esters; the large MOE (>1500) between the NOAEL for lutein equivalents in a new 13-week study and the estimated dietary exposure of 0.32 mg/kg bw per day (from additive and natural sources); a 2-fold increase in the NOAEL for lutein as a result of another new 13-week study; and the fact that lutein esters from *Tagetes erecta* are considered to be substitutional for other lutein extracts.

At its sixty-third meeting, the Committee established a group ADI of 0–2 mg/kg bw for lutein from *Tagetes erecta* and synthetic zeaxanthin. At the

present meeting, the Committee established a temporary ADI "not specified" for lutein esters from *Tagetes erecta*. The ADI was made temporary because the specifications for lutein esters from *Tagetes erecta* were tentative.

The Committee considered establishing a group ADI "not specified" for lutein esters from *Tagetes erecta* that would include lutein from *Tagetes erecta* and synthetic zeaxanthin and related xanthophylls, but this would be possible only when the specifications for lutein esters from *Tagetes erecta* are finalized.

The Committee noted that limited information was received from the sponsor on the manufacturing process for and composition of the substance. Although a single compound was claimed as the major component of the extract, it was unclear whether this was the case, as it appeared that lutein and a number of similar carotenoids could be esterified with at least three different fatty acids. The analytical data from only one final product were supplied, and these did not give details on the composition of the carotenoids, the non-carotenoid portion of the extract, including waxes, and the fatty acid–containing fraction. The Committee prepared new specifications and, in view of the above limited information, made them tentative and requested the following information by the end of 2015 to complete the safety assessment:

- details on the manufacturing process, including purification steps;
- detailed analytical data on the full composition of at least five different batches of commercially available product to support the specifications;
- method of analysis to determine carotenoid composition; and
- method of analysis to determine the composition of the non-carotenoid lipidic fraction.

A toxicological monograph was prepared.

3.1.6 Octenyl succinic acid (OSA)–modified gum arabic

At its seventy-first meeting (Annex 1, reference *196*), the Committee evaluated the toxicological, chemical and technical data for OSA-modified gum arabic. In view of the similarities between OSA-modified gum arabic and the parent gum arabic, toxicological information for gum arabic was included in the toxicological monograph. At that meeting, the Committee decided to allocate a temporary ADI "not specified" to OSA-modified gum arabic, pending submission of data by the end of 2011 showing hydrolysis of OSA-modified gum arabic in the gastrointestinal tract to confirm the validity of using toxicological data on gum arabic in the evaluation of OSA-modified gum arabic. The Committee at its seventy-first meeting prepared new specifications for OSA-modified gum arabic.

At the seventy-fourth meeting (Annex 1, reference *205*), the Committee evaluated new data on the hydrolysis of OSA-modified gum arabic and reviewed the specifications. The Committee concluded that the results from the experiments on the hydrolysis of OSA-modified gum arabic did not unequivocally demonstrate that OSA-modified gum arabic hydrolyses completely in the stomach into gum arabic and OSA. Furthermore, the hydrolysis experiments showed inconsistencies with the reported stability of OSA-modified gum arabic in food. Therefore, the Committee deferred further evaluation of OSA-modified gum arabic and requested that the following data be provided by the end of 2013:

- data resolving the concern about the stability of OSA-modified gum arabic in food;
- data confirming that OSA-modified gum arabic is (completely) hydrolysed in the gastrointestinal tract, to confirm the validity of using gum arabic in the evaluation of OSA-modified gum arabic.

The temporary ADI was retained and the specifications were revised with changes in the test methods for the degree of esterification and for residual OSA content.

At the seventy-seventh meeting (Annex 1, reference *214*), the Committee evaluated a new study on the hydrolysis of OSA-modified gum arabic in simulated gastric fluid, simulated intestinal fluid and water. The Committee noted that complete hydrolysis of OSA-modified gum arabic under neutral pH conditions in simulated intestinal fluid or water, as reported in the study, was not in accordance with the claimed stability of the OSA ester linkage in aqueous solutions at the pH range of foods and beverages. The Committee considered that the spontaneous hydrolysis of OSA-modified gum arabic in water was unlikely to occur, which therefore raised doubts about the validity of the observed hydrolysis in the presence of gastrointestinal enzymes. In view of this, the Committee considered that the study did not unequivocally demonstrate that OSA-modified gum arabic hydrolyses completely in the stomach into gum arabic and OSA and that the validity of using toxicological data on gum arabic in the evaluation of OSA-modified gum arabic had not been confirmed.

The Committee also reviewed data on the stability of OSA-modified gum arabic in food and considered that these data demonstrated that OSA-modified gum arabic provided a stable emulsion in the two model food systems evaluated. However, the data did not unequivocally demonstrate that the OSA-modified gum arabic, at the molecular level, is stable (i.e. not hydrolysed) in food and beverages.

The Committee noted that ongoing studies on the stability of OSA-modified gum arabic in food may provide further information on its chemical fate in food and aqueous solutions, which could help to explain the contradictory

results of the hydrolysis study. Therefore, the Committee decided to retain the temporary ADI "not specified" pending submission of additional data on the stability of OSA-modified gum arabic in food, by the end of 2013.

The Committee also reviewed the specifications and noted that the purity test of degree of esterification in the current specifications should be replaced by the degree of substitution and requested information for an analytical method to measure the degree of substitution and results of the analysis of at least five commercially available batches. The specifications were made tentative pending submission of these data by the end of 2013.

At the present meeting, the Committee evaluated a demulsification study in simulated gastric fluid, simulated intestinal fluid and water using emulsions prepared with OSA-modified gum arabic. The Committee also evaluated data provided for the chemical characterization of OSA-modified gum arabic, as well as the method proposed to evaluate the degree of substitution and results of the analysis of commercial batches of the product in commerce.

Biochemical data

The demulsification study was performed in simulated gastric fluid, simulated intestinal fluid and water using emulsions prepared with OSA-modified gum arabic. The stability of the emulsions was verified using backscattering analysis, and undiluted emulsions were used as controls. Backscattering analysis conducted on undiluted emulsions indicated that the emulsions were stable over time, whereas the emulsions diluted 23 times in water, simulated intestinal fluid and simulated gastric fluid started to fail (demulsification) after approximately 3–4 hours, depending on the sample *(10)*. From backscattering analysis, it is possible to verify changes in particle size or concentration as a function of time. This technique provides information about the stability of an emulsion, but its use is not appropriate to study the dissociation of OSA from the gum at a molecular level.

Evaluation

The Committee is of the opinion that the demulsification study does not provide appropriate evidence that OSA-modified gum arabic is fully hydrolysed in the gastrointestinal tract to gum arabic and OSA and that the validity of using toxicological data on gum arabic in the evaluation of OSA-modified gum arabic has not been confirmed.

The Committee received data about the chemical composition of OSA-modified gum arabic in commerce, which were not in accordance with the existing specifications. The Committee noted that the residual (free) OSA was in the range of 3–4% (weight per weight [w/w]), whereas in the specifications monograph, a

value not higher than 0.3% is specified. The Committee was informed that there is no correlation between the free OSA content and emulsion stability and that the residual OSA does not contribute to the emulsifying properties, but is present as an impurity of the manufacturing process, which uses about 3% (w/w) OSA in the synthesis. The Committee noted that the high amount of free OSA in the product in commerce is not in accordance with the amount reported to be used in the synthesis of the manufacturing process. The submitted data did not clarify the nature of the linkage of the OSA and the gum; hydrogen bonding as well as covalent bonding (ester linkage) have been suggested by the sponsor. A ^{1}H nuclear magnetic resonance (NMR) method was proposed by the sponsor to determine the ratio of OSA to gum, but data on the degree of substitution using ^{1}H NMR or another appropriate method were not provided. Owing to the high amount of free OSA in the product, data obtained using ^{1}H NMR as well as ^{13}C NMR did not prove unequivocally that the gum was esterified.

The Committee revised the specifications and maintained the tentative status pending submission of data on the manufacturing process, including purification steps; chemical characterization of the product in commerce; updated analytical methods for the determination of esterified (bound) and residual (free) OSA; results of the analysis of at least five batches of product in commerce; and applicability of the high-performance liquid chromatographic method for the determination of residual OSA.

The Committee noted that in addition to the requested information, additional safety data may be needed to complete the evaluation of OSA-modified gum arabic. The Committee decided that the temporary ADI "not specified" will be withdrawn unless adequate data to complete the safety evaluation are submitted by the end of 2015.

No addendum to the toxicological monograph was prepared.

3.1.7 Octenyl succinic acid (OSA)–modified starch (starch sodium octenyl succinate)

Explanation

OSA-modified starch (starch sodium octenyl succinate) is listed in the GSFA *(3)* for use as a stabilizer, emulsifier and thickener in several food categories. The Committee previously reviewed OSA-modified starch at its thirteenth and twenty-sixth meetings (Annex 1, references *19* and *59*). At the twenty-sixth meeting, the Committee allocated an ADI "not specified" to OSA-modified starch because the only significant finding (corticomedullary calcium deposition in the kidney) was considered to be related to a marginal magnesium deficiency when carbohydrate comprises a major portion of the diet. The Committee prepared specifications for modified starches, including OSA-modified starch, at its thirty-

fifth meeting (Annex 1, reference *88*). These specifications were later revised by the Committee at the fifty-seventh, seventy-first and seventy-fourth meetings (Annex 1, references *154*, *196* and *205*).

At the request of CCFA at its Forty-fifth Session *(9)*, the Committee evaluated the safety of OSA-modified starch for use as an emulsifier in infant formula and in formula for special medical purposes intended for infants. Data submitted for evaluation included metabolic studies in rats, dogs and infants; short-term studies in rats, dogs and pigs; a long-term study in rats; and genotoxicity studies. Data from five trials in human infants were also included, as was some information from the post-marketing surveillance on a product containing OSA-modified starch.

Chemical and technical considerations

Starch sodium octenyl succinate (INS 1450; CAS No. 66829-29-6) is obtained by the modification of food starch with OSA. This modification involves controlled esterification by the introduction of lipophilic octenyl succinic groups from *n*-octenyl succinic anhydride to waxy starch pretreated with acid. The resulting *n*-octenyl succinate ester slurry undergoes several processing steps prior to being cooked under controlled temperature and pressure and spray-dried. The final starch sodium octenyl succinate product should contain not more than 3% octenyl succinyl groups and not more than 0.3% free OSA.

Biochemical data

The results of in vitro and in vivo studies conducted in experimental animals and humans demonstrate that OSA-modified starch is at least partially hydrolysed in the gastrointestinal tract by digestive enzymes to form OSA and native starch. The starch component undergoes typical carbohydrate digestion and absorption, whereas OSA is absorbed and excreted as the unchanged compound or metabolized via a combination of ω-, ω-1 and β-oxidation steps, similar to the metabolism of other branched-chain fatty acids, and then excreted. The fates of OSA-modified starch and the hydrolysed product OSA are similar in rats, dogs and human infants with respect to enzyme hydrolysis in the digestive tract, followed by absorption, metabolism and elimination, with the only difference being the amount of OSA excreted unchanged in the urine. The clinical data indicate that infants are also able to metabolize OSA to a number of different metabolites, including propane-1,2,3-tricarboxylic acid. While the degree of metabolism may differ among species, in general, the same metabolites are produced. Therefore, results from studies in rats and dogs conducted on OSA-modified starch are considered relevant for supporting the safety assessment of OSA-modified starch in humans.

Toxicological data

At its twenty-sixth meeting, the Committee noted that in a 90-day feeding study of OSA-modified starch at dietary levels up to 30% in the rat, the only significant finding was corticomedullary calcium deposition in the kidney. The twenty-sixth meeting of the Committee also made some general observations on the toxicological relevance of nephrocalcinosis in the rat. It noted that nephrocalcinosis may arise as a consequence of a physiological influence of carbohydrate intake on mineral metabolism, which needs to be taken into account in assessing the possible toxicological relevance of nephrocalcinosis. Since that time, there have been a large number of reports of nephrocalcinosis in response to exposures to carbohydrates with no changes in renal function, demonstrating that nephrocalcinosis in rats given large amounts of carbohydrates is not relevant to humans.

In a 90-day oral toxicity study not previously evaluated by the Committee, rats were fed diets containing 30% OSA-modified starch, equal to approximately 37 000 mg/kg bw per day. No test article–related adverse effects were reported.

The lack of toxicity of OSA-modified starch is further supported by the results of a long-term study in which no signs of toxicity were reported in rats fed OSA-modified starch at concentrations of up to 30% in the diet, equal to 17 and 21 g/kg bw per day for males and females, respectively, for up to 120 weeks.

OSA-modified starch has not shown evidence of genotoxicity in vitro. In addition, long-term dietary administration of OSA-modified starch produced no evidence of carcinogenicity in rats.

There were no data available on reproductive or developmental toxicity.

Special studies in young animals

Neonatal Beagle pups, 5–9 days old, were dosed twice daily by gavage with OSA-modified starch in water at doses of 5000 or 10 000 mg/kg bw per day for 6 weeks. Each dose group consisted of four puppies of each sex and was fed by a single dam. Body weight gain was lower than expected in all groups, including both water and starch controls, in the first 3 weeks, which was attributed to limited milk supply due to large litter size. Pups in the high-dose OSA-modified starch group were less active after 15 days and had decreased body weight gain compared with controls throughout the study. This effect could not be clearly attributed to OSA-modified starch because each treatment group of eight puppies was nursed by a single dam. No other toxicologically relevant findings were reported.

A follow-up study to investigate the reported effects in the high-dose group was conducted. Three-month-old Beagle pups were fed OSA-modified starch at dietary concentrations of 5.5%, 11.0% or 22.0%, equivalent to 3000, 6000 and 12 000 mg/kg bw per day, for 42 days. No clinical or histopathological

effects (including in the kidney) were reported for any dose group, but a decrease in body weight gain was reported for the high dose group animals compared with the starch-fed control animals. The differences in body weight gain were probably due to incomplete OSA-modified starch digestion, resulting in lower caloric intake.

In a GLP-compliant 3-week toxicity study conducted in neonatal piglets, OSA-modified starch was administered at doses of 1000, 2000 or 10 000 mg/kg bw per day via a feeding device 6 times per day. The piglets were administered OSA-modified starch for 3 weeks starting 2 days after birth, to model the 0- to 12-week period of development in human infants for which infant formula may be provided as the sole source of nutrition. OSA-modified starch was well tolerated in piglets and did not produce any definitive compound-related effects on growth or the clinical pathology parameters evaluated. No effects attributable to the test article were observed upon macroscopic or histopathological evaluation. As the digestive systems of neonatal swine and human infants are similar, the results of this study are relevant to the safety assessment of OSA-modified starch for use in infant formula.

Human studies

A single dose of 25 g of OSA-modified starch was well tolerated by fasting healthy non-diabetic adults and attenuated the post-prandial glycaemic response compared with glucose.

Two randomized, multicentre, double-blind clinical studies have been conducted to investigate the effects of infant formula supplemented with OSA-modified starch. In the first study, infants (approximately 50 per group) were fed either control formula or formula reported to contain OSA-modified starch as the sole source of nutrition at 1.33–1.47 g/100 mL beginning from 2–16 days of age for 120 days. No effects on growth were noted. There was no difference between the groups in illnesses or "symptoms of concern" in the infants as reported by the parents. In the second study, in which 168 infants 0–8 days of age were fed two similar casein hydrolysate formulas both containing less than 2% OSA-modified starch for comparison with commercially available control formula until day 28 of life, tolerance of the infants to OSA-modified starch formula was also examined. No issues with tolerability were reported with the OSA-modified starch formula.

In addition, two growth studies in which OSA-modified starch formula (concentration not specified) was included as the control formula have been evaluated. In these studies, 165 and 289 infants were fed OSA-modified starch containing formula from 14 through 120 days of age, and normal healthy growth and tolerance were reported. In a third growth study, 213 infants 0–9 days of age were fed infant formula containing OSA-modified starch at 16 g/L (1.6%),

calculated to give an OSA-modified starch exposure of approximately 2.5 g/kg bw per day through 112 days of age. Overall, the formula containing OSA-modified starch was well tolerated, and no health-related concerns were reported.

Post-marketing surveillance information on a recently globally marketed infant formula containing 2% OSA-modified starch to be used for special medical purposes has indicated that OSA-modified starch is well tolerated when administered to infants as recommended.

Assessment of dietary exposure

OSA-modified starch is proposed for use in infant formula and formula for special medical purposes intended for infants at levels up to 20 g/L formula.

Median infant formula consumption estimates were derived from the estimated energy requirements for fully formula-fed infants. It should be noted that the energy requirements of formula-fed infants are greater than those of breastfed infants, although this disparity decreases with increasing age. A further exposure scenario was considered using high (95th percentile) daily energy intakes reported for formula-fed infants. The highest reported 95th percentile energy intakes were for infants aged 14–27 days. For all dietary exposure estimates, a common energy density of formula of 67 kcal/100 mL (280 kJ/100 mL) was used to convert energy to the volume of formula ingested daily. The maximum proposed use level results in median estimated exposures to OSA-modified starch of up to 3.7 g/kg bw per day in infants aged 0–6 months, whereas infants with high (95th percentile) energy intakes may reach exposure levels of 4.4 g/kg bw per day.

Evaluation

At the twenty-sixth meeting, the Committee assigned an ADI "not specified" to OSA-modified starch. Since the time of that meeting, new data have become available, including a 90-day oral toxicity study, genotoxicity studies and a long-term toxicity and carcinogenicity study. All of the new data confirm the very low toxicity of OSA-modified starch, and the Committee confirmed the ADI "not specified".

Several new studies submitted were relevant to assessing the safety of OSA-modified starch in infant formula and formula for special medical purposes intended for infants.

Of the two studies conducted in neonatal animals, the study in piglets was considered the more relevant. The NOAEL of OSA-modified starch was 10 g/kg bw per day, the highest dose tested. The MOEs based on this NOAEL are 2.3 for the infants with the 95th percentile of energy intake (4.4 g OSA-modified starch/kg bw per day) and 2.7 at the median energy intake (3.7 g OSA-modified starch/kg bw per day).

In addition, several studies in human infants have shown that OSA-modified starch at concentrations up to 2% in infant formula is well tolerated; an exposure of 2.5 g/kg bw per day was provided for one of these studies. Post-marketing surveillance of an infant formula containing 2% OSA-modified starch also confirmed that it was well tolerated by infants.

The Committee took into account the overall low toxicity of OSA-modified starch, the conservatism in the NOAEL, which was the highest dose tested in a study in neonatal animals, and in the exposure assessments, as well as the supporting evidence from clinical trials and post-marketing surveillance and concluded that the consumption of OSA-modified starch in infant formula or formula for special medical purposes intended for infants is not of concern at use levels up to 20 g/L.

The Committee reviewed the existing specifications for OSA-modified starch. The Committee discussed limits on lead specifications for this and the other food additives for use in infant formula on the agenda, as described in section 2.4.1. The Committee revised the specifications monograph for modified starches, amending the analytical method for octenyl succinyl groups in starch sodium octenyl succinate in the monograph.

The Committee also discussed the fact that this food additive is part of the existing specifications monograph for modified starches along with 15 other modified starches. The Committee noted that it is difficult to revise individual specifications for any given modified starch within this specifications monograph. The Committee therefore recommended that the specifications monograph for the modified starches be split into 16 individual monographs.

A consolidated toxicological monograph was prepared.

3.1.8 Paprika extract

Explanation

At its fifty-fifth meeting in 2000 (Annex 1, reference *149*), the Committee concluded that paprika oleoresin is acceptable for use as a spice. This conclusion confirmed the outcome of an evaluation performed by the Committee at its fourteenth meeting in 1970 (Annex 1, reference *22*), which stated that the product was derived from a widely consumed natural foodstuff and there were no data indicative of a toxic hazard. The use of paprika oleoresin as a spice was considered to be self-limiting and obviated the need for an ADI. Paprika extract was placed on the agenda of the sixty-ninth meeting in 2008 (Annex 1, reference *190*) at the request of the Thirty-ninth Session of CCFA *(11)* for assessment of its safety as a food colour, specifications and dietary exposure. CCFA asked if the existing safety assessment and specifications for paprika oleoresin for use as a spice could be extended to the use as a food colour. As the source material and

the manufacturing process differ for paprika preparations used as a spice and as a food colour, the name "paprika extract" was adopted for use as a food colour.

At the sixty-ninth meeting, the Committee evaluated the use of paprika extract as a food colour. A toxicological monograph and new specifications were prepared. However, the safety assessment was not completed and the new specifications were made tentative pending the receipt of additional information on paprika extract. The Committee requested information on the concentrations of capsaicin present in the extracts and additional information about the composition of batches of extract produced by a variety of manufacturers. The Committee also wanted assurance that the material tested in the 90-day and long-term studies in rats was representative of all commercial production of paprika extract. At the seventy-seventh meeting of the Committee (Annex 1, reference *214*), following receipt of additional analytical data, the specifications were revised, and the tentative status was removed. The Chemical and Technical Assessment prepared at the sixty-ninth meeting was also revised to include the composition of commercial preparations.

At the present meeting, the Committee reconsidered the toxicological data and the dietary exposure in order to establish an ADI for paprika extract for use as a food colour. These data are presented in detail in the toxicological monograph prepared at the sixty-ninth meeting and are summarized only briefly here. No new toxicity data or data on dietary exposure have been submitted to the Committee since its sixty-ninth meeting.

Chemical and technical considerations

Information on the composition of six extracts selected from different producers of commercial paprika extracts was submitted by the sponsor for the seventy-seventh meeting of the Committee in 2013 (Annex 1, reference *214*). These samples originated from India (one), Spain (four) and Peru (one). The content of total carotenoids in these extracts of paprika varied between 4.2% and 8.4%. The major carotenoid in these extracts was capsanthin, which constituted between 36.7% and 56.1% of total carotenoids. Other major carotenoid pigments were (13*Z*)-β-carotene (6.6–10.7%), (15*Z*)-β-carotene (4.2–11.9%) and zeaxanthin (3.0–12.4%). There were smaller amounts of other carotenoids, including capsorubin, which constituted between 0.6% and 2.3% of total carotenoids. Together, these are referred to as total carotenoids present in paprika extract. Levels of capsaicin were below the limit of detection (30 mg/kg) in five of the six samples and were present in the sixth sample at less than 100 mg/kg. Levels of all capsaicinoids were below 200 mg/kg (0.02% of the substance). At the seventy-seventh meeting, the Committee set the maximum content of capsaicinoids at 200 mg/kg and the maximum content of arsenic at 1 mg/kg. In addition to the

principal colour components, paprika extract also contains neutral lipids and phospholipids composed of a variety of saturated and unsaturated fatty acids. The additional data did not reveal the presence of impurities (including heavy metals and solvents) that would raise concerns for consumer safety. The composition of the paprika extract tested in the 90-day and long-term toxicity studies in rats was comparable to the composition of the extracts used in commerce as a food colour.

Biochemical data

The systemic bioavailability of carotenoids present in paprika preparations was investigated in two human studies. In one study, capsanthin, the major carotenoid present in a paprika oleoresin administered orally as a single dose to nine volunteers, was not present in the chylomicron fraction of whole blood between 1 and 12 hours after dosing. Other carotenoids (zeaxanthin, β-carotene and β-cryptoxanthin) reached a maximum concentration in blood at 6 hours *(12)*. In another study, 8 hours after four male volunteers ingested paprika juice (single dose equivalent to 34.2 μmol capsanthin), capsanthin was detected in the plasma at 0.1–0.29 μmol/L. After the volunteers ingested paprika juice 3 times daily for 1 week (daily dose equivalent to 16.2 μmol capsanthin), plasma concentrations of capsanthin reached a plateau of 0.1–0.12 μmol/L in 2–7 days *(13)*. The results indicate that the systemic bioavailability of capsanthin is considered to be low.

Toxicological data

In a 13-week study, F344 rats were given paprika extract with a carotenoid content of 7.5% and a capsaicin content of less than 0.01% *(14)*. The paprika extract was administered at dietary levels of 0% (basal diet), 0.62%, 1.25%, 2.5% and 5%. No significant adverse effects were observed at any dose level. The NOAEL was 5% in the diet, the highest concentration tested, equal to 2950 and 3200 mg/kg bw per day for males and females, respectively.

In a combined 52-week toxicity and 104-week carcinogenicity study, rats were given diets containing 0%, 2.5% or 5% paprika extract (a carotenoid content of 7.5% and a capsaicin content of <0.01%, as in the 13-week study described above). All animals were subjected to a complete pathological evaluation. No evidence of adverse effects, including carcinogenicity, was observed at either dose level or time point. The NOAEL was considered to be 5% in the diet, equal to 2388 and 2826 mg/kg bw per day (52 weeks) and 2052 and 2324 mg/kg bw per day (104 weeks) for males and females, respectively *(15)*.

The Committee noted that mutagenicity/genotoxicity tests conducted on extracts of chili peppers and samples of capsaicin have yielded mixed, inconsistent and often contradictory results. However, results of more recent studies with

pure capsaicin support the conclusion that it is not genotoxic in standard in vitro and in vivo assays. Furthermore, Hallagan and co-workers *(16)* concluded that a weight-of-evidence analysis indicates that paprika is not genotoxic, which is in agreement with the negative results of the 104-week carcinogenicity study in rats.

The Committee noted that reproductive and developmental toxicity studies for paprika extract were not available.

Assessment of dietary exposure

There are limited data on the potential dietary exposure to total carotenoids from use of paprika extract as a food colour. Dietary exposures to total carotenoids from paprika extract were based on consumption data from the United Kingdom and France. Assuming that 7% of paprika extract was total carotenoids, the estimated mean dietary exposure to total carotenoids for the French population was 2.3 mg/day, and the estimated dietary exposure for a high consumer was 7.0 mg/day. For the United Kingdom, survey data (based on four age categories) yielded mean estimated dietary exposures to total carotenoids from paprika extract of 2.9–6.9 mg/day. The exposure at the 95th percentile was estimated to be 6.3–13.2 mg/day. Exposure to capsaicin and all capsaicinoids from paprika extract is considered to be less than 0.05 mg/day, based on the low levels of all capsaicinoids present in paprika extract used as a food colour.

Evaluation

The results of a 90-day dietary toxicity study and a combined 52-week toxicity and 104-week carcinogenicity study in rats, both conducted with a paprika extract representative of the various extracts used in commerce as a food colour, did not provide evidence of any adverse effects. The NOAEL for paprika extract in both studies was 5% in the diet, the highest concentration tested, equal to 2052 mg/kg bw per day (calculated by the authors for the 104-week carcinogenicity study).

The Committee noted that the weight of evidence obtained in genotoxicity studies indicates that paprika extract is not genotoxic.

Although reproductive and developmental toxicity studies for paprika extract were not available, the Committee noted that the major components of paprika extracts were mixtures of neutral lipids and phospholipids containing saturated and unsaturated fatty acids, which are unlikely to present a reproductive or developmental hazard. Also, the Committee was aware of the results of a GLP-compliant developmental toxicity study in rats that had been performed with lutein (administered at 10% in the diet), a carotenoid of similar structure to capsanthin *(17)*. No developmental abnormalities were observed in the study with lutein at any dose level, and a NOAEL of 1000 mg/kg bw per day, the highest dose tested, was identified. Thus, the Committee concluded that paprika

extract meeting the specifications for use as a food colour is unlikely to pose a reproductive or developmental hazard.

The total carotenoid level in the paprika extracts used in the toxicity studies described above was 7.5%, which is in the mid-range of the levels in commercial paprika extracts used as colours. The NOAEL of 2052 mg/kg bw per day is equivalent to 153 mg/kg bw per day for paprika extracts when expressed as total carotenoids. The Committee applied an uncertainty factor of 100 to the NOAEL of 153 mg/kg bw per day and established an ADI for paprika extract used as a food colour of 0–1.5[4] mg/kg bw, expressed as total carotenoids.

The assessment of dietary exposure to paprika extract used as a colour was based on exposure to total carotenoids in paprika extract. Based on survey data, the highest exposure at the 95th percentile was estimated to be 6.3–13.2 mg/day (equivalent to 0.1–0.2 mg/kg bw per day, based on a body weight of 60 kg), which is below the ADI. The Committee concluded that dietary exposure to paprika extract used as a food colour does not present a health concern.

No addendum to the toxicological monograph was prepared, and the specifications were maintained.

3.1.9 Pectin

Explanation

Pectins (INS 440; CAS No. 9000-69-5) are used as a gelling, thickening and stabilizing agent and are approved for use in general foods all over the world. Pectins as food additives have been evaluated by the Committee at its thirteenth, seventeenth, eighteenth, nineteenth and twenty-fifth meetings (Annex 1, references *19*, *32*, *35*, *38* and *56*). At its twenty-fifth meeting in 1981 (Annex 1, reference *56*), the Committee established a group ADI "not specified" for pectin and amidated pectin.

Current specifications for pectins were established by the Committee at its seventy-first meeting (Annex 1, reference *198*), superseding the previous specifications set by the Committee at its sixty-eighth meeting (Annex 1, reference *189*).

At the present meeting, the Committee was asked to consider the safety of using non-amidated pectin in infant formula and formula for special medical purposes intended for infants. The Committee reviewed new data published since the twenty-fifth meeting, in particular data of relevance to the products being considered. The data submitted for evaluation included studies using pectin: a biotransformation study in rats, a study in neonatal pigs and a human infant study. In addition, a number of studies used pectin-derived oligosaccharides,

[4] The Committee noted that although derived values, such as health-based guidance values, should be rounded to a single significant figure, it decided to use two significant figures in the present case, as the impact of rounding to one significant figure would be more than 30%.

which the sponsors considered relevant to the evaluation of pectin. These studies were short-term toxicity studies in rats, a reproductive toxicity study in rats, genotoxicity studies and four studies in human infants.

Chemical and technical considerations

Pectin is a complex heteropolysaccharide that consists mainly of the partial methyl esters of polygalacturonic acid and their sodium, potassium, calcium and ammonium salts. It is obtained by aqueous extraction of appropriate edible plant material, usually citrus fruits or apples. The average molecular weight of food-grade pectin will vary depending upon the pectin source and processing and is expected to range from 100 to 200 kDa. Amidated pectin is prepared by treatment of pectin with ammonia under alkaline conditions. The existing specifications for pectins cover both pectin and amidated pectin.

Pectin is used in infant formula as a thickener to increase the viscosity of the formula and as a stabilizer to maintain the homogeneity of the formula throughout its shelf life. According to the sponsor, amidated pectin is not used in infant formula.

The Committee was made aware that a further pectin product is available on the market. This product, known as pectin-derived acidic oligosaccharides (pAOS), is produced by enzymatic hydrolysis of pectin. pAOS has not been evaluated by the Committee and is not covered by the existing specifications for pectins.

Biochemical data

Pectin is a non-digestible carbohydrate that is extensively fermented by the microflora in the gastrointestinal tract to oligogalacturonic acids, which are then further metabolized to short-chain fatty acids, such as acetate, propionate and butyrate.

pAOS is a product of the digestion of food-grade pectin and consists of small polymers predominantly of molecular weight of no more than 3800 Da. Manufactured pAOS is similar to products formed from pectin in the gastrointestinal tract. The Committee agreed that data from studies on pectin-derived oligosaccharides can support conclusions reached on the basis of data from studies that have tested pectin.

Toxicological data

The toxicity of pAOS was studied in a one-generation study in rats in which there was a 13-week investigation of F_1 offspring. Male and female parental (F_0) animals were fed test and control diets starting 4 weeks prior to mating and throughout the mating, gestation and lactation periods until weaning of the

offspring (F_1 rats). There were four diets used in the study. Two were control diets, one being the standard rodent diet plus 10% potato starch and the other a reference control diet that was the standard rodent diet plus 10% short-chain fructo-oligosaccharides, which mimic the neutral oligosaccharides of human milk. The test diets contained 5% and 10% pAOS. The calculated intakes of pAOS in the F_0 females during the premating and gestation periods were 3.1 and 6.2 g/kg bw per day in the 5% and 10% pAOS groups, respectively. The overall intake of pAOS during the early phases of the testing up to the end of lactation was up to 7.1 g/kg bw per day.

No treatment-related effects were seen in clinical signs, body weights, growth rate, feed intake or reproductive indices in the dams. Pups were also unaffected in their general condition or on histopathological examination. In the subsequent subchronic phase of the study with rats selected from the F_1 generation, effects attributable to the test substance were increased urinary sodium concentration and excretion and urinary pH in males in both dose groups and increased urinary sodium excretion and urinary pH in females in the 10% group. The study authors attributed the increased sodium excretion to high sodium levels (3120 mg/100 g) in pAOS. Caecum weights were increased in the 5% and 10% dose groups, and kidney weights were also increased in males at the 10% dose. Taking into account that caecum weight increases are commonly observed with high-fibre diets in rats and that there was no indication of disturbed renal function or kidney histopathology, the Committee concluded that these effects were of no toxicological relevance. In addition, an increase in diffuse hyperplasia of the bladder epithelium was seen in males and females of the high-dose group, with the effect reaching statistical significance only in males.

A 13-week study in rats was carried out to investigate the cause of the effects seen in the above study and to determine a NOAEL. The dose groups were 1%, 2.5% and 10% pAOS; in addition, there was an additional group fed test material containing 10% pAOS and ammonium chloride to test whether acidification of the urine could prevent the diffuse hyperplasia of the bladder epithelium. The overall intakes of pAOS, with and without ammonium chloride, in high-dose rats were 7.2 and 7.1 g/kg bw per day, respectively. The overall intakes of pAOS in the 1% and 2.5% dose groups were 0.7 and 1.7 g/kg bw per day, respectively. Diffuse hyperplasia of the bladder epithelium was again seen in males of the 10% pAOS group, and a similar change, albeit less prominent, was observed in females of this group. Very slight diffuse hyperplasia of the bladder epithelium was also noted in one female of the 1% pAOS group, but not at the next higher dose level, and this finding was therefore considered incidental. Urinary sodium excretion was increased in rats fed 10% pAOS, with and without ammonium chloride, and the urinary sodium concentration was higher in males than in females. The incidence and severity of the diffuse hyperplasia of the bladder epithelium

in this study were lower than in the first study. Rats fed pAOS in combination with ammonium chloride had lower urinary pH, and diffuse hyperplasia of the bladder epithelium was not observed. The authors commented that this finding showed that the hyperplasia was caused by the concurrent increase in urinary sodium concentration and pH, a condition known to predispose rats to hyperplasia of the bladder epithelium, and not to pAOS itself. Diffuse hyperplasia of the bladder epithelium resulting from a concurrent increase in urinary pH and urinary sodium ion concentration is a well known phenomenon in rats and not frequently observed in other animal species. On this basis, the Committee agreed that because of the species specificity of the response, diffuse hyperplasia of the bladder epithelium induced by this mechanism in rats is considered not to be of relevance to humans; the Committee concluded that the NOAEL of pAOS was about 7 g/kg bw per day, the highest dose tested.

There were no new data available on long-term toxicity or carcinogenicity.

The genotoxicity of pAOS was examined in a bacterial reverse mutation assay, a mouse lymphoma assay, a chromosome aberration study in Chinese hamster ovary cells and a rat micronucleus test. The bacterial reverse mutation assay with and without metabolic activation was negative. Positive responses were seen in the other two in vitro studies only in the absence of metabolic activation and in the presence of significant cytotoxicity. The mouse lymphoma assay showed a positive response at 2920 μg/mL and an equivocal response at 3590 μg/mL (the highest concentration tested). Cytotoxicity was observed at these concentrations, and the results were determined to be equivocal. The chromosome aberration study indicated that the positive response and cytotoxicity were related to the solvent (dimethyl sulfoxide), and the authors concluded that pAOS was cytotoxic, but not clastogenic. No increases in micronuclei were observed in bone marrow polychromatic erythrocytes of rats fed pAOS at approximately 7 g/kg bw per day in the one-generation study described above. There was no change in the ratio of normochromatic to polychromatic erythrocytes. Overall, the Committee concluded that pAOS is not genotoxic.

In the one-generation study in rats dosed with pAOS, there was no effect seen on reproduction or on general condition of the pups, litter size, pup viability or sex ratio.

Special studies in young animals

Neonatal piglets were administered pectin in milk replacer formula as their sole source of nutrition for 3 weeks after birth at a concentration of 0.5, 3.0 or 10.0 g/L (doses calculated to be 142, 847 and 3013 mg/kg bw per day for males and 141, 879 and 3094 mg/kg bw per day for females, respectively). The milk replacer formula was offered to the piglets 6 times per day at a dose volume of 500 mL/kg

bw, and the intake of pectin was determined from the amount of milk replacer formula consumed per day. Decreased intake of the milk replacer was correlated with mean decreased body weights in males in the 10.0 g/L dose group (3.88 kg versus 4.35 kg in controls) from day 13, which reached statistical significance (5.22 kg versus 6.47 kg in controls) at termination (day 21). For females, the mean body weights were consistently lower in the 10.0 g/L dose group from day 15 (4.3 kg versus 4.5 kg in controls), and at termination they were 5.65 kg compared with 5.95 kg in controls. Feed consumption in males in the 10.0 g/L dose group was decreased by 30% by the end of the study. The body weights in the two lowest dose groups were similar to those of controls. There were minor changes seen in haematology tests, but they were not considered to be toxicologically relevant. Caecum and colon weights were statistically significantly increased in the top two dose groups.

No treatment-related histopathological changes were seen in the intestinal tract. This study showed that pectin in doses up to 847 mg/kg bw per day in milk replacer administered to neonatal pigs over a 3-week period was well tolerated. A decreased intake of the milk replacer and an associated decrease in body weight were seen at a pectin dose of 3013 mg/kg bw per day.

Human studies

One study has investigated the effects of pectin in preterm human infants. Infants (n = 74) receiving human milk fortified with a liquid infant milk fortifier (stated by the sponsor to contain 0.085% pectin after addition to the human milk) for 28 days were compared with infants (n = 72) receiving human milk fortified with a control milk fortifier. At the conclusion of the study, the pectin-fed infants had a significantly higher linear growth rate, along with greater increases in weight, length and head circumference. No treatment-related adverse events were observed. These results suggest that pectin at 0.085% in infant formula was well tolerated in preterm infants.

Four studies investigated the effects of infant formula containing pAOS on term human infants. In one study, a cohort of healthy infants (n = 414) enrolled in the study at ages 20–42 days was fed an infant formula containing 1.2 g/L (0.12%) pAOS until the age of 12 months. No differences were reported in growth, gastrointestinal tolerance or stool frequency between infants fed the pAOS formula and infants fed a control formula, but stool consistency was softer in infants fed the pAOS formula. A second study showed that pAOS did not affect growth characteristics in healthy infants (n = 27) when they consumed a formula containing 0.2% pAOS daily (240 mg/kg bw per day) for 2 months.

In the last two studies, the effect of pAOS on gut microflora in infants was investigated. In one study, term infants (n = 16) were given an infant formula

containing 0.2% pAOS every day for 6 weeks. Microbiological analysis of faecal samples from the group fed pAOS infant formula indicated no effect on the counts of *Bifidobacterium* sp., *Clostridium* sp., *Escherichia coli* or *Enterobacter* sp. In the final study, in which term infants (n = 82) were fed an infant formula containing 0.2% pAOS (equivalent to 240 mg/kg bw per day), there was no effect on the same bacteria as in the previous study compared with those receiving a control formula.

Assessment of dietary exposure

The maximum proposed use level of non-amidated pectin in formula intended for infants aged 0–12 weeks is 5 g/L.

Median infant formula consumption estimates were derived from estimated energy requirements for fully formula-fed infants. It should be noted that the energy requirements of formula-fed infants are greater than those of breastfed infants, although this disparity decreases with increasing age. A further exposure scenario was considered, using high (95th percentile) daily energy intakes reported for formula-fed infants. The highest reported 95th percentile energy intakes were for infants aged 14–27 days. For all dietary exposure estimates, a common energy density of formula of 67 kcal/100 mL (280 kJ/100 mL) was used to convert energy to the volume of formula ingested daily.

The proposed use level for pectin in infant formula results in median estimated exposures to pectin of 0.75–0.91 g/kg bw per day in infants ages 0–12 weeks, whereas infants with high (95th percentile) energy intakes may reach an exposure level of 1.1 g/kg bw per day.

Evaluation

At the twenty-fifth meeting, the Committee assigned an ADI "not specified" to pectin and amidated pectin. At the present meeting, the Committee reviewed new data that were particularly of relevance to the safety assessment of the use of non-amidated pectin in infant formula and formula for special medical purposes intended for infants. Many of the data reviewed by the Committee were from studies that had examined pectin-derived oligosaccharides such as pAOS. As manufactured pAOS is similar to products formed from pectin in the gastrointestinal tract, the Committee concluded that these studies were relevant to the evaluation of pectin in infant formula.

In short-term toxicity studies, feeding test material containing pAOS to rats for 13 weeks was reported to cause diffuse hyperplasia of the bladder epithelium, which was considered to be species specific and not relevant to humans. The NOAEL of pAOS in these studies was about 7 g/kg bw per day, the highest dose tested.

Genotoxicity studies on pAOS gave negative or equivocal results in vitro and a negative result in vivo following 90 days of administration in the rat at doses up to about 7 g/kg bw per day. The Committee concluded that pAOS is not genotoxic.

In a 3-week study in neonatal pigs fed pectin-containing milk replacer, the NOAEL of pectin was 847 mg/kg bw per day, with decreased feed intake and body weight gain observed at 3013 mg/kg bw per day. The Committee concluded that the neonatal pig is an appropriate model for the human infant. Using the NOAEL from this study, the MOEs were estimated to be 0.9 for infants with median energy intake and 0.8 for infants with high (95th percentile) energy intake.

In human infant studies, one showed that pectin in infant formula was well tolerated by preterm infants at a concentration of 0.085%. Four studies with pAOS (at up to 0.2%) in formulas provided some support for the tolerance of infants to pectin.

The Committee concluded that estimated exposure to pectin from its use in infant formula is in the region of the NOAEL of pectin derived from the neonatal pig study (847 mg/kg bw per day) and close to the LOAEL based on decreased feed intake and body weight gain. Although no overt toxicological effects were observed in the neonatal pigs, decreased food intake and body weight gain would be considered an undesirable effect in human infants. The available clinical studies were mainly conducted with pectin or pectin-derived oligosaccharides at concentrations of 0.2% or less and therefore do not provide support for tolerance and normal growth at the maximum proposed use level. Therefore, the Committee concluded that the use of pectin in infant formulas at the maximum proposed use level (0.5%) is of concern.

The Committee requests additional data to support the safety evaluation of pectin in infant formula, including an explanation for the decreased feed intake and body weight gain in neonatal pigs.

The Committee at its seventy-first meeting (2009) had prepared specifications for pectins. The Committee discussed limits on lead specifications for this and the other food additives for use in infant formula that were on the agenda, as described in section 2.4.1. At the present meeting, the specifications for pectins were maintained.

An addendum to the toxicological monograph was prepared.

3.2 Revision of specifications

3.2.1 Citric acid

Citric acid was on the agenda at the present meeting at the request of the Forty-fifth Session of CCFA *(9)* due to an inconsistency in the limit test for oxalate in its specifications monograph. The current provisions of the oxalate limit test include a maximum value for absorbance that does not represent the limit specified for oxalic acid. The test method in the Compendium of Food Additive Specifications, FAO JECFA Monographs 1, Volume 4 (Annex 1, reference *180*), was revised (see section 2.4.3). The revised method will be published in the Compendium of Food Additive Specifications, FAO JECFA Monographs 16 (2014).

The specifications for citric acid were revised with a reference to the revised method.

3.2.2 Gellan gum

Gellan gum was placed on the agenda of the present meeting at the request of the Forty-fifth Session of CCFA *(9)* to consider the use of ethanol in the manufacturing process as an alternative to 2-propanol. The specifications were revised accordingly, and a revised method for the determination of residual solvents using headspace gas chromatography was included. The assay method in the specifications refers to the alginates assay method in Volume 4 of the Combined Compendium of Food Additive Specifications (Annex 1, reference *180*), which was replaced with a new method without the use of mercury (see section 2.4.2).

3.2.3 Polyoxyethylene (20) sorbitan monostearate

The Committee at its twenty-fifth meeting in 1981 (Annex 1, reference *56*) had prepared specifications for polyoxyethylene (20) sorbitan monostearate. At its Forty-fifth Session *(9)*, CCFA requested a revision of the specifications, in particular a change in the saponification and hydroxyl values. Based on the analytical data provided, the Committee revised the specifications to incorporate the proposed changes.

3.2.4 Potassium aluminium silicate

PAS was on the agenda of the present meeting at the request of the Forty-sixth Session of CCFA *(2)* for consideration of deleting the functional use of carrier in the specifications monograph for PAS. According to Codex *(18)*, a carrier is "a food additive used to dissolve, dilute, disperse or otherwise physically modify a food additive or nutrient without altering its function (and without exerting any technological effect itself) in order to facilitate its handling, application or

use of the food additive or nutrient". At its seventy-seventh meeting (Annex 1, reference *214*), the Committee revised the specifications for PAS and included "carrier (used as a carrier substrate in pearlescent pigments made with titanium dioxide and/or iron oxide)" as one of its functional uses. At the present meeting, the Committee reviewed the existing data as well as new information received from the sponsor and noted that PAS stabilizes the formed layers of titanium dioxide and/or iron oxide in the PAS-based pearlescent pigments. Therefore, the Committee concluded that PAS exerts a technological effect in the PAS-based pearlescent pigments; as a result, PAS could not be considered to function as a carrier according to the Codex definition. Hence, the Committee decided to delete the functional use as carrier in the specifications monograph for PAS.

The specifications were revised accordingly.

3.2.5 *Quillaia* extract (Type 2)

The Committee at its sixty-fifth meeting in 2005 (Annex 1, reference *178*) had prepared specifications for *Quillaia* extract (Type 2). At its Forty-fifth Session *(9)*, CCFA requested a revision of the specifications concerning the upper limit in the loss on drying specification, from 80% to 90%. Based on the analytical data submitted, the Committee revised the specifications to incorporate the proposed change.

4. Flavouring agents

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

Assignment to structural class

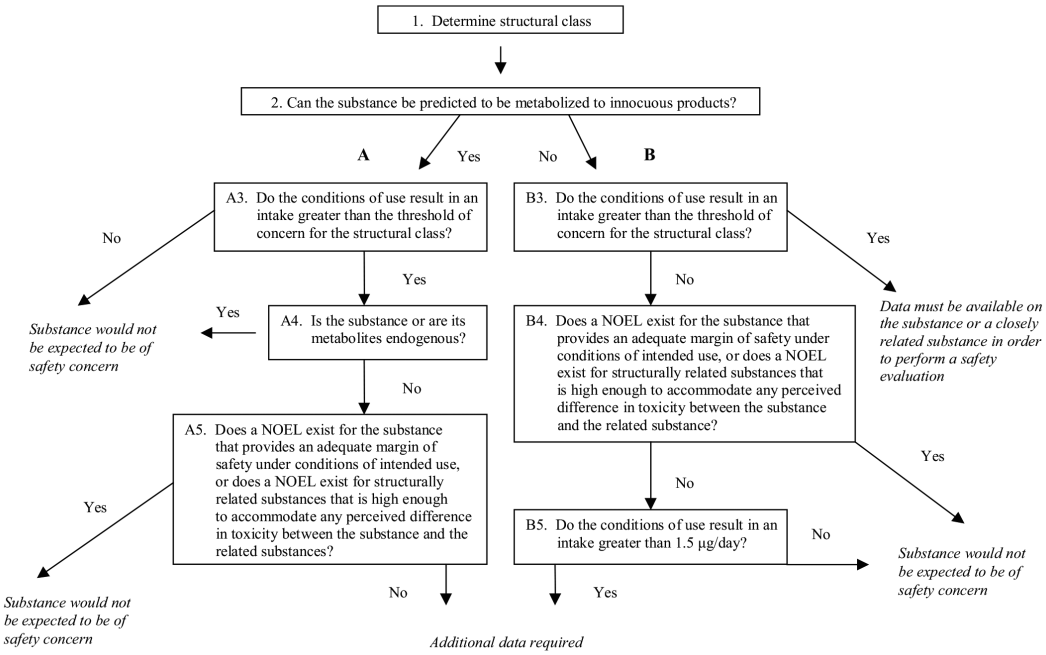
Eight groups of flavouring agents were evaluated using the Procedure for the Safety Evaluation of Flavouring Agents, as outlined in Fig. 1 (Annex 1, references *116*, *122*, *131*, *137*, *143*, *149*, *154*, *160*, *166*, *173* and *178*). In applying the Procedure, the chemical is first assigned to 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 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.

Figure 1
Procedure for the Safety Evaluation of Flavouring Agents



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 41×10^6 in Europe, 13×10^6 in Japan and 31×10^6 in the USA.[5]

[5] Population counts in 2010 were reported by IOFI *(19)* to be 410 million for Europe (EU-16 plus Turkey and Switzerland), 309 million for the USA and 128 million for Japan.

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:

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

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 website at http://www.who.int/foodsafety/publications/jecfa/en/.

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 alicyclic hydrocarbons

Introduction

The Committee evaluated six additional flavouring agents belonging to the group of aliphatic and alicyclic hydrocarbons. The additional flavouring agents included five aliphatic alkenes (Nos 2191, 2192, 2194, 2195 and 2196) and one alicyclic hydrocarbon (No. 2197). The Committee decided not to evaluate α-ionene (No. 2193), which was also submitted for evaluation as part of this group, because it determined that the chemical structure of this aromatic hydrocarbon does not fit into the aliphatic and alicyclic hydrocarbons group. The evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents. None of these flavouring agents has previously been evaluated by the Committee.

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 were of no safety concern at estimated dietary exposures. One member of this group, *d*-limonene (No. 1326), was previously evaluated by the Committee at its thirty-ninth meeting (Annex 1, reference *101*) and was assigned an ADI of 0–1.5 mg/kg bw. At its forty-first meeting (Annex 1, reference *107*), the Committee re-evaluated the ADI for *d*-limonene and recommended that it be withdrawn and replaced with an ADI "not specified". At the sixty-third meeting, the ADI "not specified" was maintained for *d*-limonene (Annex 1, reference *173*).

Three of the six flavouring agents in this group (Nos 2191, 2192 and 2195) have been reported to occur naturally in foods. They have been detected in, for example, apples, citrus fruits, mushrooms, peanuts, walnuts, cheese, eggs, milk, honey, beef, pork and chicken.

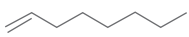
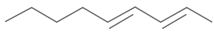
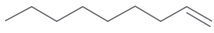
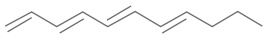
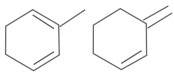
Assessment of dietary exposure

The total annual volume of production of the six flavouring agents belonging to the group of aliphatic and alicyclic hydrocarbons is approximately 0.2 kg in Europe, 2380 kg in the USA and 0.4 kg in Japan. More than 99% of the annual production volume in the USA is accounted for by 4-methyl-*cis*-2-pentene (No. 2194). Half of the annual production volume in Japan is accounted for by 1-nonene (No. 2195). The volume of the annual production in Europe is equally accounted for by 1-octene (No. 2191) and 2,4-nonadiene (No. 2192).

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 1. The estimated dietary exposure is highest for 4-methyl-*cis*-2-pentene (No. 2194) (263 µg/day, MSDI value). For the other flavouring agents, the estimated dietary exposures, calculated using either the SPET or

Table 1

Summary of the results of the safety evaluations of aliphatic and alicyclic hydrocarbons 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					
1-Octene	2191	111-66-0 	No, SPET: 3	Note 1	No safety concern
2,4-Nonadiene	2192	56700-78-8 	No, SPET: 13	Note 1	No safety concern
4-Methyl-*cis*-2-pentene	2194	691-38-3 	No, MSDI: 263	Notes 1 and 2	No safety concern
1-Nonene	2195	124-11-8 	No, SPET: 0.6	Note 1	No safety concern
1,3,5,7-Undecatetraene	2196	116963-97-4 	No, SPET: 0.6	Note 1	No safety concern
Mixture of methyl cyclohexadiene and methylene cyclohexene	2197	30640-46-1; 1888-90-0 	No, SPET: 3	Notes 1 and 3	No safety concern

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

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

[c] *Step 2*: The six 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/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, except for No. 2194, for which the MSDI estimate was higher.

Notes:

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

the MSDI method, range from 0.01 to 13 μg/day, 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 aliphatic and alicyclic hydrocarbons has previously been described in the monograph from the sixty-third meeting (Annex 1, reference *174*). Additional information on the metabolism of 1-octene (No. 2191) was available for this 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 above-mentioned flavouring agents, the Committee assigned all six flavouring agents to structural class I *(6)*.

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

Step A3. The highest estimated dietary exposures to all six flavouring agents in this group are below the threshold of concern (i.e. 1800 μg/day for class I). The Committee therefore concluded that these flavouring agents would not pose a safety concern at current estimated dietary exposures.

Table 1 summarizes the evaluations of the six flavouring agents belonging to this group of aliphatic and alicyclic hydrocarbons (Nos 2191, 2192 and 2194–2197).

Consideration of combined intakes from use as flavouring agents

The six additional flavouring agents in this group of aliphatic and alicyclic hydrocarbons have MSDI values of 0.01–263 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 (No. 2192) has a minimum assay value of less than 95% (see Annex 3). The secondary components of 2,4-nonadiene (No. 2192) are 1,3-nonadiene, 2,6-nonadiene and 2,7-nonadiene. These compounds, which are structurally related to No. 2192, are considered not to present a safety concern at estimated dietary exposures from use of No. 2192 as a flavouring agent.

Conclusion

In the previous evaluation of flavouring agents in this group of aliphatic and alicyclic hydrocarbons, studies of biochemistry, acute toxicity, short-term and long-term toxicity and genotoxicity were available (Annex 1, reference *174*). None of the 20 flavouring agents of this group raised safety concerns.

For the present evaluation, biochemical data were available for one flavouring agent in this group (No. 2191). For previously evaluated flavouring agents in this group, a study of acute toxicity (No. 1324), studies of short-term toxicity (Nos 1327 and 1324), studies of long-term toxicity (No. 1327) and studies of genotoxicity (Nos 1324, 1327, 1329, 1336, 1339 and 1341) were available. Also, a study of acute toxicity was available for methyl cyclohexadiene, a constituent of No. 2197, which also contains the structurally related methylene cyclohexene. The studies available for the present evaluation support the previous safety evaluations.

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

An addendum to the toxicological monograph was prepared.

4.1.2 Aliphatic and aromatic ethers

Introduction

The Committee evaluated three flavouring agents belonging to the group of aliphatic and aromatic ethers. The flavouring agents included two cyclic ethers (Nos 2137 and 2189) and one phenyl ether (No. 2190). The evaluations were conducted using the Procedure for the Safety Evaluation of Flavouring Agents (Annex 1, reference *131*). One of these flavouring agents (No. 2137) was previously evaluated by the Committee at its seventy-sixth meeting (Annex 1, reference *211*). The two others (Nos 2189 and 2190) have not previously been evaluated by the Committee.

The Committee previously evaluated 29 other members of this group of flavouring agents at its sixty-first meeting (Annex 1, reference *166*). The Committee concluded that all 29 flavouring agents in that group were of no safety concern at estimated dietary exposures.

The Committee also evaluated 10 additional members of this group of flavouring agents at its seventy-sixth meeting (Annex 1, reference *212*). The Committee concluded that nine of these flavouring agents were of no safety concern at estimated dietary exposures. For one flavouring agent, nerolidol oxide (No. 2137), additional data were required to complete the evaluation.

None of the flavouring agents in this group has been reported to occur naturally in food.

Assessment of dietary exposure

The total annual volumes of production of the three aliphatic and aromatic ethers are approximately 20 kg in the USA and 0.3 kg in Japan. Approximately 75% of the total annual volume of production in the USA is accounted for by one flavouring agent in this group – 1-cyclopropanemethyl-4-methoxybenzene (No. 2190).

Dietary exposures were estimated using the MSDI method and the SPET. The highest estimated dietary exposure for each flavouring agent is reported in Table 2. The estimated daily dietary exposure is highest for nerolidol oxide (No. 2137) (2500 µg/day, the SPET value obtained from frozen dairy products). For the other flavouring agents, daily dietary exposures ranged from 0.08 to 1250 µg/day, with the SPET yielding the highest estimate in each case.

Absorption, distribution, metabolism and elimination

Information on the absorption, distribution, metabolism and elimination of flavouring agents belonging to the group of aliphatic and aromatic ethers has previously been described (Annex 1, references *167* and *212*). No additional information was available for this 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 above-mentioned flavouring agents, the Committee assigned all three flavouring agents (Nos 2137, 2189 and 2190) to structural class III *(6)*.

Step 2. All three flavouring agents in this group are predicted to be metabolized to innocuous products. The evaluation of these substances 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/day for class III). Accordingly, the evaluation of all three flavouring agents proceeded to step A4.

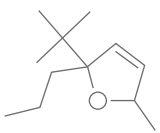
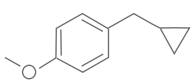
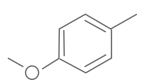
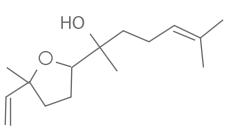
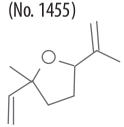
Step A4. None of the three flavouring agents or their metabolites are endogenous substances. Accordingly, the evaluation of all three flavouring agents proceeded to step A5.

Step A5. For cassyrane (No. 2189), a NOAEL of 50 mg/kg bw per day from a 28-day oral gavage study in rats provides an MOE of 2400 in relation to the highest estimated dietary exposure to No. 2189 (SPET = 1250 µg/day or 21 µg/kg bw per day) when used as a flavouring agent. The Committee therefore concluded that cassyrane (No. 2189) would not pose a safety concern at current estimated dietary exposures.

For 1-cyclopropanemethyl-4-methoxybenzene (No. 2190), a NOAEL for the structurally related substance *p*-methylanisole (No. 1243) of 40 mg/kg bw per

Table 2

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 A4* Is the flavouring agent or are its metabolites endogenous?	*Step A5*[e] Adequate margin of exposure for the flavouring agent or related substances?	Comments on predicted metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
Structural class III								
Cassyrane	2189	871465-49-5 	Yes, SPET: 1250	No	Yes. The NOAEL of 50 mg/kg bw per day for cassyrane in a 28-day oral gavage study in rats is at least 2400 times the estimated daily dietary exposure to No. 2189 when used as a flavouring agent.	Note 1	–	No safety concern
1-Cyclopropane-methyl-4-methoxybenzene	2190	16510-27-3 	Yes, SPET: 360	No	Yes. The NOAEL of 40 mg/kg bw per day for the structurally related *p*-methylanisole in a 28-day oral gavage study in rats is at least 6700 times the estimated daily dietary exposure to No. 2190 when used as a flavouring agent.	Notes 2 and 3	*p*-Methylanisole (No. 1243) 	No safety concern
Nerolidol oxide	2137	1424-83-5 	Yes, SPET: 2500	No	Yes. The NOAEL of 103 mg/kg bw per day for the structurally related anhydrolinalool oxide in a 90-day dietary study in rats is at least 2500 times the estimated daily dietary exposure to No. 2137 when used as a flavouring agent.	Note 1	Anhydrolinalool oxide (No. 1455) 	No safety concern

[a] The evaluations of 38 of 39 flavouring agents in this group were previously completed by the Committee (Annex 1, references *166* and *211*).

[b] *Step 1*: All three flavouring agents are in structural class III.

[c] *Step 2*: All three flavouring agents are expected 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 MOE was calculated based on the estimated dietary exposure calculated by the SPET.

Notes:

1. Oxidized by cytochrome P450 isoenzymes to polar metabolites, followed by conjugation with glucuronic acid and elimination in the urine.
2. Metabolized by cytochrome P450–mediated *O*-demethylation.
3. Metabolized by cytochrome P450–mediated ring hydroxylation.

day from a 28-day oral gavage study in rats provides an MOE of 6700 in relation to the highest estimated dietary exposure to No. 2190 (SPET = 360 μg/day or 6 μg/kg bw per day) when used as a flavouring agent. The Committee therefore concluded that 1-cyclopropanemethyl-4-methoxybenzene (No. 2190) would not pose a safety concern at current estimated dietary exposures.

For nerolidol oxide (No. 2137), a NOAEL for the structurally related substance anhydrolinalool oxide (No. 1455) of 103 mg/kg bw per day from a 90-day dietary study in rats provides an MOE of 2500 in relation to the highest estimated dietary exposure to No. 2137 (SPET = 2500 μg/day or 42 μg/kg bw per day) when used as a flavouring agent. The Committee therefore concluded that nerolidol oxide (No. 2137) would not pose a safety concern at current estimated dietary exposures.

Table 2 summarizes the evaluations of the three flavouring agents belonging to the group of aliphatic and aromatic ethers (Nos 2137, 2189 and 2190).

Consideration of combined intakes from use as flavouring agents

The three flavouring agents in this group of aliphatic and aromatic ethers have low MSDI values (0.08–2 μg/day). The Committee concluded that consideration of combined intakes is not necessary, because these flavouring agents would not contribute significantly to the combined intake of this group.

Conclusion

In the previous evaluations of flavouring agents in the 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 (Annex 1, references *167* and *212*). For one flavouring agent, nerolidol oxide (No. 2137), additional data were required to complete the evaluation.

For the present evaluation, additional acute toxicity studies (Nos 2189 and 2190), short-term studies of toxicity (No. 2189) and genotoxicity studies (Nos 2189 and 2190) were available. For previously evaluated flavouring agents, additional studies on short-term toxicity (Nos 1234 and 1237) and genotoxicity (No. 1237) were available.

The Committee concluded that these three flavouring agents, two of 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.

An addendum to the toxicological monograph was prepared.

4.1.3 Ionones and structurally related substances

Introduction

The Committee evaluated three additional flavouring agents belonging to the group of ionones and structurally related substances. The additional flavouring agents included an ionone, β-isomethylionone (No. 2186); an acyclic ionone analogue, pseudoionone (No. 2187); and a damascone, *trans*-α-damascone (No. 2188). The evaluations were conducted using the Procedure for the Safety Evaluation of Flavouring Agents (Annex 1, reference *131*). None of these flavouring agents has previously been evaluated by the Committee.

The Committee previously evaluated allyl-α-ionone at its twenty-fourth meeting (Annex 1, reference *53*) and concluded that there were inadequate data to establish an ADI. A further three members of the group were considered at the twenty-eighth meeting (Annex 1, reference *66*), when a group ADI of 0–0.1 mg/kg bw was established. At the Committee's fifty-first meeting (Annex 1, reference *137*), an additional 21 members of this group of flavouring agents were considered. For 20 of these flavouring agents, the Committee concluded that they would not give rise to safety concerns based on the estimated dietary exposures. The remaining substance (No. 402) was not considered to be sufficiently similar to the structural characteristics of the group and was not evaluated further.

One of the three flavouring agents considered at the current meeting, pseudoionone (No. 2187), is a natural component of food and has been detected in liquorice, yerba maté tea, passionfruit juice, tamarind, Chinese microbial-fermented tea and tomato at levels up to 5 mg/kg.

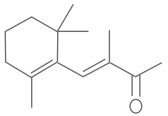
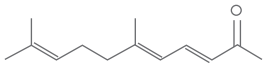
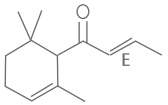
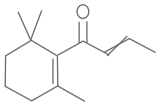
Assessment of dietary exposure

The total annual volumes of production of the three flavouring agents belonging to the group of ionones and structurally related substances are 1 kg in Europe, 3 kg in the USA and 1 kg in Japan. More than 66% of the annual production volume in the USA is accounted for by pseudoionone (No. 2187). More than 99% of the annual production volume in Europe and Japan is accounted for by *trans*-α-damascone (No. 2188).

Dietary exposures were estimated using both the SPET and the MSDI method, with the highest values reported in Table 3. The highest estimated dietary exposure is for pseudoionone (No. 2187) (1000 μg/day, the SPET value obtained from gelatines and puddings). For the other flavouring agents, dietary exposures as SPET or MSDI estimates range from 0.01 to 600 μg/day, with the SPET yielding the highest estimate in each case.

Table 3

Summary of the results of the safety evaluations of ionones and structurally related substances 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 B4*[e] Adequate margin of exposure for the flavouring agent or related substances?	*Step B5* Do the conditions of use result in an intake >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 I								
β-Isomethylionone	2186	79-89-0 	A3: No, SPET: 600	NR	NR	Notes 1 and 2	–	No safety concern
Pseudoionone	2187	141-10-6 	A3: No, SPET: 1000	NR	NR	Note 2	–	No safety concern
trans-α-Damascone	2188	24720-09-0 	B3: No, SPET: 600	The NOAEL of 2 mg/kg bw per day for the structurally related β-damascone (No. 384) in a 90-day study in rats is 200 times the SPET estimate and 500 000 times the MSDI when No. 2188 is used as a flavouring agent.	Yes	Note 1	β-Damascone (No. 384) 	Additional data required to complete evaluation

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] Twenty flavouring agents in this group were previously evaluated by the Committee (Annex 1, reference *137*).

[b] *Step 1*: The three flavouring agents are in structural class I (Nos 2186–2188).

[c] *Step 2*: Nos 2186 and 2187 can be predicted to be metabolized to innocuous products. No. 2188 cannot be predicted to be metabolized to innocuous products.

[d] The threshold for human dietary exposure for structural class I is 1800 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 MOEs were calculated based on the estimated dietary exposure calculated using the SPET. In cases where the resulting MOE was relatively low, a comparison with the MSDI was also made.

Notes:

1. Metabolized primarily via ring oxidation to 3-oxo and 3-hydroxy derivatives, which are eliminated.
2. Metabolized primarily through reduction of the ketone followed by conjugation with glucuronic acid; alternatively, Michael addition to the alkene group by glutathione followed by elimination as the mercapturic acid conjugate.

Absorption, distribution, metabolism and elimination

The absorption, metabolism and elimination of flavouring agents of the ionones and structurally related substances group have previously been described (Annex 1, reference *138*). Orally administered ionones are absorbed and metabolized in mammals by allylic hydroxylation of the ring followed by oxidation of the hydroxyl group to corresponding ketone derivatives. Reduction of the ketone function to the corresponding secondary alcohol also occurs. Combinations of these detoxication reactions result in the formation of multiple polar metabolites that are excreted in the urine unchanged or conjugated with glucuronic acid.

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 three flavouring agents (Nos 2186, 2187 and 2188) to structural class I *(6)*.

Step 2. Two of the flavouring agents (Nos 2186 and 2187) in this group are predicted to be metabolized to innocuous products. The evaluation of these flavouring agents therefore proceeded via the A-side of the Procedure. The other flavouring agent (No. 2188) in this group cannot be predicted to be metabolized to innocuous products. Therefore, the evaluation of this flavouring agent proceeded via the B-side of the Procedure.

Step A3. The highest estimated daily dietary exposures for each of the two flavouring agents in structural class I that are predicted to be metabolized to innocuous products (Nos 2186 and 2187) are below the threshold of concern (i.e. 1800 µg/day for class I). The Committee therefore concluded that neither of the two flavouring agents would pose a safety concern at current estimated dietary exposures.

Step B3. The highest estimated daily dietary exposure for the flavouring agent in structural class I that is not predicted to be metabolized to innocuous products (No. 2188) is below the threshold of concern (i.e. 1800 µg/day for class I). Accordingly, the evaluation of this flavouring agent proceeded to step B4.

Step B4. For *trans*-α-damascone (No. 2188), the NOAEL of 2 mg/kg bw per day for the structurally related β-damascone (No. 384) in a 90-day study in rats is 200 times the SPET estimate (600 µg/day or 10 µg/kg bw per day) and 500 000 times the MSDI (0.3 µg/day or 0.004 µg/kg bw per day) when No. 2188 is used as a flavouring agent. The Committee therefore concluded that the NOAEL does not provide an adequate MOE based on the SPET, and the evaluation proceeded to step B5.

Step B5. The conditions of use result in an intake greater than 1.5 µg/day. Therefore, the Committee concluded that additional data are required to complete the evaluation.

Table 3 summarizes the evaluations of the three ionones and structurally related substances used as flavouring agents (Nos 2186, 2187 and 2188) in this group.

Consideration of combined intakes from use as flavouring agents

The two additional flavouring agents in this group of ionones and structurally related substances that were concluded to be of no safety concern have low MSDI values (0.01–0.2 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 group.

Conclusion

In the previous evaluation of flavouring agents in this group of ionones and structurally related substances, studies of acute toxicity, short-term toxicity and genotoxicity were available (Annex 1, reference *138*). None of the 20 previously evaluated flavouring agents raised safety concerns.

For the present evaluation, studies of acute toxicity (No. 2187), studies of genotoxicity (Nos 2187 and 2188) and a study of developmental toxicity (No. 2187) were available. For previously evaluated flavouring agents, there were additional studies of acute toxicity (Nos 388, 389, 394, 399 and 404), studies of short-term toxicity (No. 404), studies of genotoxicity (Nos 386–389, 394, 401, 403 and 404) and studies of developmental toxicity (Nos 389 and 404). The additional data provided supported the previous safety evaluations.

The Committee concluded that two of these three flavouring agents (Nos 2186 and 2187), which are additions to the group of ionones and structurally related substances evaluated previously, would not give rise to safety concerns at current estimated dietary exposures. For *trans*-α-damascone (No. 2188), the Committee requires additional toxicological and/or dietary exposure information in order to complete the evaluation. The Committee was aware of additional genotoxicity data reporting equivocal results for a structurally related damascone; therefore, information to address any concerns regarding potential genotoxicity should also be provided.

An addendum to the toxicological monograph was prepared.

4.1.4 Miscellaneous nitrogen-containing substances

Introduction

The Committee evaluated two additional flavouring agents belonging to the group of miscellaneous nitrogen-containing substances. These flavouring agents were a uridic diamide with additional ester functionality and an alicyclic alkyl side-chain (No. 2203) and an aminoquinoline carboxylic acid derivative containing an aliphatic amide side-chain (No. 2204). The evaluations were conducted using

the Procedure for the Safety Evaluation of Flavouring Agents (Annex 1, reference *131*). The two flavouring agents have not previously been evaluated by the Committee, and both are reported to be flavour modifiers.

The Committee previously evaluated 16 other members of this group of flavouring agents at the sixty-fifth meeting (Annex 1, reference *178*), 14 other members of this group at the sixty-ninth meeting (Annex 1, reference *190*) and 2 other members of this group at the seventy-sixth meeting (Annex 1, reference *211*). The Committee concluded that all 32 of these flavouring agents were of no safety concern at estimated dietary exposures.

Neither of the additional flavouring agents in this group (Nos 2203 and 2204) has been reported to occur naturally in food.

Assessment of dietary exposure

The total annual volume of production for the two flavouring agents belonging to the group of miscellaneous nitrogen-containing substances is 10 kg in the USA, with no reported data from Europe or Japan. Ninety per cent of the annual production volume in the USA is accounted for by 4-amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid (No. 2204).

Dietary exposures were estimated using both the SPET and the MSDI method, with highest values reported in Table 4. The estimated dietary exposure is highest for 4-amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid (No. 2204) (2400 μg/day, the SPET value obtained from non-alcoholic beverages). For the other flavouring agent, 3-[3-(2-isopropyl-5-methylcyclohexyl)-ureido]-butyric acid ethyl ester (No. 2203), the MSDI was 0.1 μg/day, and the SPET value was 800 μg/day.

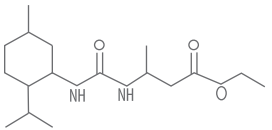
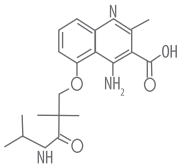
Absorption, distribution, metabolism and elimination

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, sixty-ninth and seventy-sixth meetings (Annex 1, references *179*, *191* and *212*).

Two common metabolic pathways are expected to be involved in the metabolism of 3-[3-(2-isopropyl-5-methylcyclohexyl)-ureido]-butyric acid ethyl ester (No. 2203). As an ethyl ester, it will be hydrolysed by carboxylesterases in gastric juice, intestinal fluid and hepatocytes. Released carboxylic acids will be excreted in urine either free or conjugated with glucuronic acid or glycine. As an alkyl *N*-substituted urea, No. 2203 is also expected to be oxidized at the α-carbon group by cytochrome P450 enzymes to generate an unstable carbinol urea, which is expected to release the corresponding ketones, menthone and 3-oxobutyric acid ethyl ester.

Table 4

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 estimated dietary exposure exceed the threshold of concern?	*Follow-on from step B3*[e] Are additional data available for the 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-[3-(2-Isopropyl-5-methylcyclohexyl)-ureido]-butyric acid ethyl ester	2203	1160112-20-8 	Yes, SPET: 800	Yes. The NOAEL of 776.5 mg/kg bw per day in a 90-day study in rats is 60 000 times the estimated dietary exposure to No. 2203 when used as a flavouring agent.	Note 1	No safety concern
4-Amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropox)-2-methylquinoline-3-carboxylic acid (and its hemisulfate monohydrate salt)	2204	1359963-68-0 	Yes, SPET: 2400	Yes. The NOAEL of 100 mg/kg bw per day in a 90-day study in rats is 2500 times the estimated dietary exposure to No. 2203 when used as a flavouring agent.	Note 2	No safety concern

[a] Thirty-two flavouring agents in this group were previously evaluated by the Committee (Annex 1, references *178*, *190* and *211*).

[b] *Step 1*: The two flavouring agents in this group (Nos 2203 and 2204) 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 values listed represent the highest daily dietary exposures calculated by either the SPET or the MSDI method. The SPET gave the highest estimated dietary exposure in each case.

[e] The MOEs were calculated based on the estimated dietary exposure calculated by the SPET.

Notes:

1. The ester functionality will readily hydrolyse to the corresponding acid and ethanol, which will form conjugates with glucuronic acid and be readily eliminated. The ureido functionality will be oxidized by cytochrome P450 and form the α-carbinol. The carbinol urea is expected to release urea and the corresponding ketones, menthone and 3-oxobutyric acid ethyl ester.
2. Primarily excreted in the faeces unchanged, with very minor amounts of oxidative metabolites eliminated in the urine.

Metabolic studies for 4-amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid (No. 2204) have shown that this compound and its hemisulfate monohydrate salt have low oral bioavailability in rats. Once absorbed, the parent compound is poorly metabolized, being primarily excreted unchanged in the faeces, with only 0.1–0.6% eliminated in the urine as possible metabolites. In a study in which human or rat liver microsomes were incubated with 4-amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid (No. 2204) for 60 minutes, less than 0.1% was converted to oxidative metabolites. Thus, either very little or no Phase 1 metabolism occurred.

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 both flavouring agents (Nos 2203 and 2204) to structural class III *(6)*.

Step 2. Neither of the flavouring agents in this group (Nos 2203 and 2204) can be predicted to be metabolized to innocuous products. The evaluation of both of these flavouring agents proceeded via the B-side of the Procedure.

Step B3. The highest estimated dietary exposures for both flavouring agents in structural class III are above the threshold of concern (i.e. 90 μg/day for class III). Accordingly, data must be available 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:

In accordance with the Procedure, additional data were evaluated for 3-[3-(2-isopropyl-5-methylcyclohexyl)-ureido]-butyric acid ethyl ester (No. 2203) and 4-amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid (No. 2204), as their estimated dietary exposures exceeded the threshold of concern for structural class III (90 μg/day).

For 3-[3-(2-isopropyl-5-methylcyclohexyl)-ureido]-butyric acid ethyl ester (No. 2203), a genotoxicity study and a 90-day toxicity study were available. This flavouring agent was negative for bacterial mutagenesis with and without exogenous activation. The NOAEL of 776.5 mg/kg bw per day (the highest dose tested) in a 90-day study in rats provides an MOE of 60 000 (SPET = 800 μg/day or 13 μg/kg bw per day) when No. 2203 is used as a flavouring agent. The Committee concluded that, on the basis of all of the available evidence, 3-[3-(2-isopropyl-5-methylcyclohexyl)-ureido]-butyric acid ethyl ester (No. 2203) would not pose a safety concern at current estimated dietary exposures.

For 4-amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid (No. 2204), pharmacokinetic data and 28-day and 90-day toxicity, genotoxicity and developmental toxicity studies were available. This flavouring agent was found to be poorly bioavailable and rapidly excreted in the faeces unchanged. A NOAEL of 100 mg/kg bw per day was identified in a 28-day and a 90-day study in rats. The flavouring agent was negative for bacterial mutagenesis with and without an exogenous activation system, for clastogenicity in human blood lymphocytes with and without an exogenous activation system and for induction of micronuclei in mouse bone marrow erythrocytes. The developmental toxicity study in rats had a NOAEL of 1000 mg/kg bw per day, the highest dose tested. The NOAEL of 100 mg/kg bw per day in the 90-day study in rats provides an MOE of 2500 (SPET = 2400 μg/day or 40 μg/kg bw per day) when No. 2204 is used as a flavouring agent. The Committee concluded that, on the basis of all of the available evidence, 4-amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid and its hemisulfate monohydrate salt would not pose a safety concern at current estimated dietary exposures.

Table 4 summarizes the evaluations of the two flavouring agents belonging to the group of miscellaneous nitrogen-containing substances (Nos 2203 and 2204).

Consideration of combined intakes from use as flavouring agents

The two additional flavouring agents in this group of miscellaneous nitrogen-containing substances have low MSDI values (0.1–1 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 evaluations of flavouring agents in this group of miscellaneous nitrogen-containing substances, studies of acute toxicity, short-term toxicity and genotoxicity were available (Annex 1, references *179*, *191* and *212*).

For the present evaluation, additional biochemical data, short-term studies of toxicity (28–90 days), in vitro and in vivo genotoxicity studies and a developmental toxicity study were available for the two additional flavouring agents belonging to this group (Nos 2203 and 2204).

The Committee concluded that these two flavouring agents, 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.

4.1.5 Monocyclic and bicyclic secondary alcohols, ketones and related esters

Introduction

The Committee evaluated four additional flavouring agents belonging to the group of monocyclic and bicyclic secondary alcohols, ketones and related esters. The additional flavouring agents included one bicyclic secondary alcohol, 2,2,6,7-tetramethylbicyclo[4.3.0]nona-4,9(1)-dien-8-ol (No. 2198); and three bicyclic ketones, *dl*-camphor (No. 2199), *l*-fenchone (No. 2200) and 2,2,6,7-tetramethylbicyclo[4.3.0]nona-4,9(1)-dien-8-one (No. 2201). The evaluations were conducted using the Procedure for the Safety Evaluation of Flavouring Agents (Annex 1, reference *131*). None of these flavouring agents has previously been evaluated by the Committee.

The Committee previously evaluated 32 other members of this group of flavouring agents at its sixty-third meeting (Annex 1, reference *173*). The Committee concluded that all 32 flavouring agents in that group were of no safety concern at estimated dietary exposures.

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

None of the flavouring agents in this group has been reported to occur as a natural component of food.

Assessment of dietary exposure

The total annual volumes of production of the four flavouring agents belonging to the group of monocyclic and bicyclic secondary alcohols, ketones and related esters are 491 kg in Europe, 0.3 kg in the USA and 30 kg in Japan. More than 99% of the total annual volumes of production in Europe and Japan is accounted for by *dl*-camphor (No. 2199).

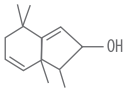
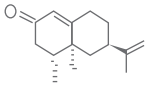
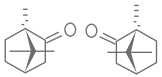
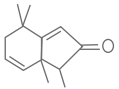
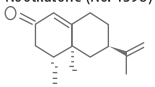
Dietary exposures were estimated using both the SPET and the MSDI method, with the highest values reported in Table 5. The estimated dietary exposures are highest for 2,2,6,7-tetramethylbicyclo[4.3.0]nona-4,9(1)-dien-8-ol (No. 2198) and 2,2,6,7-tetramethylbicyclo[4.3.0]nona-4,9(1)-dien-8-one (No. 2201) (625 µg/day, the SPET value obtained from jams and jellies). For the other flavouring agents, the estimated dietary exposures range from 0.01 to 150 µg/day, with the SPET yielding the highest estimates in all cases.

Absorption, distribution, metabolism and elimination

Information on the absorption, distribution, metabolism and elimination of flavouring agents of the monocyclic and bicyclic secondary alcohols, ketones and related esters group has previously been described in the reports of the sixty-

Table 5

Summary of the results of the safety evaluations of monocyclic and bicyclic secondary alcohols, ketones 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 related substances?	Comments on predicted metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
Structural class II								
2,2,6,7-Tetramethyl-bicyclo[4.3.0]nona-4,9(1)-dien-8-ol	2198	97866-86-9 	Yes, SPET: 625	No	Yes. The NOAEL of 10 mg/kg bw per day for the structurally related nootkatone in a 28-day study in rats is 1000 times the estimated dietary exposure to No. 2198 when used as a flavouring agent.	Notes 1 and 2	Nootkatone (No. 1398) 	No safety concern
dl-Camphor	2199	76-22-2 	No, SPET: 150	No	NR	Notes 1, 2 and 3	–	No safety concern
l-Fenchone	2200	7787-20-4 	No, SPET: 3	No	NR	Notes 1, 2 and 3	–	No safety concern
2,2,6,7-Tetramethyl-bicyclo[4.3.0]nona-4,9(1)-dien-8-one	2201	97844-16-1 	Yes, SPET: 625	No	Yes. The NOAEL of 10 mg/kg bw per day for the structurally related nootkatone in a 28-day study in rats is 1000 times the estimated dietary exposure to No. 2201 when used as a flavouring agent.	Notes 1, 2 and 3	Nootkatone (No. 1398) 	No safety concern

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

[b] *Step 1:* The four flavouring agents in this group (Nos 2198–2201) are in structural class II.

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

[d] The threshold for human dietary exposure for structural class II is 540 µg/day. All dietary exposure values are expressed in µg/day. The dietary exposure value listed represents the highest estimated dietary exposure calculated by either the SPET or the MSDI method. The SPET gave the highest estimated dietary exposure in each case.

[e] The MOEs were calculated based on the estimated dietary exposure calculated by the SPET.

Notes:

1. Formation of glucuronic acid conjugates directly or after metabolism, which are subsequently eliminated in the urine.
2. Cytochrome P450–mediated oxidation of alkyl ring substituents and ring positions.
3. Reduced to yield the corresponding alcohol.

third and sixty-ninth meetings (Annex 1, references *174* and *191*). New data on a representative member of this group show 20% bioavailability of orally administered D-borneol (No. 1385) in mice.

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 four flavouring agents (Nos 2198–2201) to structural class II *(6)*.

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

Step A3. The estimated dietary exposures for two of the flavouring agents (Nos 2199 and 2200) in structural class II are below the threshold of concern (i.e. 540 µg/day for class II). According to the Procedure, the safety of these flavouring agents raises no concern at current estimated dietary exposures. The estimated dietary exposures for two flavouring agents (Nos 2198 and 2201) are above the threshold of concern for structural class II (i.e. 540 µg/day for class II). Accordingly, the evaluation of these flavouring agents proceeded to step A4.

Step A4. These flavouring agents (Nos 2198 and 2201) and their metabolites are not endogenous, and therefore their evaluations proceeded to step A5.

Step A5. For 2,2,6,7-tetramethylbicyclo[4.3.0]nona-4,9(1)-dien-8-ol (No. 2198), the NOAEL of 10 mg/kg bw per day for the structurally related substance nootkatone (No. 1398) obtained in a 28-day study in rats provides an adequate MOE of 1000 in relation to the estimated dietary exposure to No. 2198 (SPET = 625 µg/day or 10 µg/kg bw per day) when used as a flavouring agent. The Committee therefore concluded that 2,2,6,7-tetramethylbicyclo[4.3.0]nona-4,9(1)-dien-8-ol (No. 2198) would not pose a safety concern at current estimated dietary exposures.

For 2,2,6,7-tetramethylbicyclo[4.3.0]nona-4,9(1)-dien-8-one (No. 2201), the NOAEL of 10 mg/kg bw per day for the structurally related substance nootkatone (No. 1398) obtained in a 28-day study in rats provides an adequate MOE of 1000 in relation to the estimated dietary exposure to No. 2201 (SPET = 625 µg/day or 10 µg/kg bw per day) when used as a flavouring agent. The Committee therefore concluded that 2,2,6,7-tetramethylbicyclo[4.3.0]nona-4,9(1)-dien-8-one (No. 2201) would not pose a safety concern at current estimated dietary exposures.

Table 5 summarizes the evaluations of the four monocyclic and bicyclic secondary alcohols, ketones and related esters used as flavouring agents (Nos 2198–2201) in this group.

Consideration of combined intakes from use as flavouring agents

The four additional flavouring agents in this group of monocyclic and bicyclic secondary alcohols, ketones and related esters have low MSDI values (0.01–41 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 group.

Conclusion

In the previous evaluations of flavouring agents in this group of monocyclic and bicyclic secondary alcohols, ketones and related esters, studies of acute toxicity, short-term and long-term toxicity and genotoxicity were available. For previously evaluated substances of this group, additional biochemical data were available at this meeting (No. 1385); a short-term study of toxicity (No. 1867), studies of genotoxicity (Nos 1385 and 1867) and a study of reproductive and developmental toxicity (No. 1388) were also available. The toxicity data available for this evaluation supported those from the previous evaluations (Annex 1, references *174* and *191*).

The Committee concluded that these four flavouring agents (Nos 2198–2201), which are additions to the group of monocyclic and bicyclic secondary alcohols, ketones and related esters, would not give rise to safety concerns at current estimated dietary exposures.

An addendum to the toxicological monograph was prepared.

4.1.6 Phenol and phenol derivatives

Introduction

The Committee evaluated four flavouring agents belonging to the group of phenol and phenol derivatives. The additional flavouring agents included a flavanone (No. 2207), a dihydrochalcone (No. 2208), a polyphenol (No. 2209) and a lignan (No. 2210). The evaluations were conducted using the Procedure for the Safety Evaluation of Flavouring Agents (Annex 1, reference *131*). These four flavouring agents have not previously been evaluated by the Committee, and all 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 *150*), 13 other members at its seventy-third meeting (Annex 1, reference *203*) and 3 additional members at its seventy-sixth meeting (Annex 1, reference *212*). The Committee concluded that all 64 flavouring agents were of no safety concern at estimated dietary exposures.

Two of the four flavouring agents (Nos 2207 and 2210) in this group have been reported to occur naturally and can be found in a broad variety of

fruits, vegetables, grains, nuts, seeds, coffee and tea, in addition to many other foods.

Assessment of dietary exposure

The total annual volumes of production of the four flavouring agents in the phenol and phenol derivatives group are approximately 1 kg in the USA and 721 kg in Japan. Approximately 96% of the total annual volume of production in Japan is accounted for by one flavouring agent in this group, myricitrin (No. 2207).

Dietary exposures were estimated using the SPET and the MSDI method, with the highest values reported in Table 6. The estimated dietary exposure is highest for (–)-matairesinol (No. 2210) (7500 μg/day, the SPET value for non-alcoholic beverages). For the other flavouring agents, the dietary exposures ranged from 0.03 to 6000 μg/day, with the SPET yielding the highest estimate in each case.

Absorption, distribution, metabolism and elimination

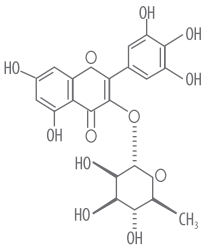
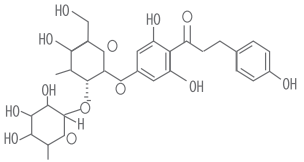
Information on the absorption, distribution, metabolism and elimination of the flavouring agents belonging to the phenol and phenol derivatives group has previously been described in the monographs of the fifty-fifth, seventy-third and seventy-sixth meetings (Annex 1, references *150*, *203* and *212*); additional information on the absorption, distribution, metabolism and elimination of polyphenols was also available for this meeting.

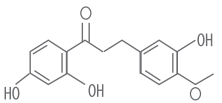
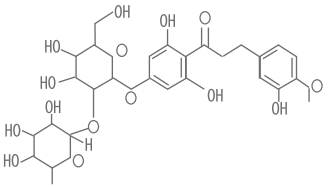
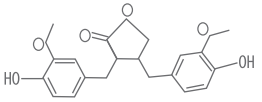
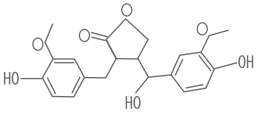
Glycoside conjugates of polyphenols are hydrolysed on the brush border of small intestine epithelial cells or within the epithelial cells. Polyphenols are rapidly but incompletely absorbed after oral administration. Metabolism occurs both in the gastrointestinal tract and after absorption. Absorbed polyphenols are metabolized through hydrolysis, sulfation, glucuronidation and/or methylation. Urinary excretion of parent substance or metabolites is rapid to relatively slow. Biliary excretion also occurs. Metabolites not absorbed in the small intestine may undergo further metabolism in the large intestine. Both glycosylated and aglycone metabolites may be excreted in the faeces. The microflora may also cleave conjugated moieties, with the resultant aglycones undergoing ring fission, leading to phenolic acid and cinnamic acid derivatives. These metabolites may 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 above-mentioned flavouring agents, the Committee assigned one flavouring agent (No. 2207) to structural class II and three flavouring agents (Nos 2208–2210) to structural class III *(6)*.

Table 6
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 margin of exposure for the flavouring agent or related substances?	Comments on predicted metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
Structural class II								
Myricitrin	2207	17912-87-7 	Yes, SPET: 3000	No	Yes. The NOAEL of 884 mg/kg bw per day in a 52-week study in rats is 18 000 times the estimated dietary exposure to No. 2207 when used as a flavouring agent.	Notes 1 and 2	–	No safety concern
Structural class III								
Naringin dihydrochalcone	2208	18916-17-1 	Yes, SPET: 4000	No	Yes. The NOAEL of 500 mg/kg bw per day in short-term studies in rats is 7500 times the estimated dietary exposure to No. 2208 when used as a flavouring agent.	Notes 1 and 2	–	No safety concern

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 related substances?	Comments on predicted metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
1-(2,4-Dihydroxyphenyl)-3-(3-hydroxy-4-methoxyphenyl)-propan-1-one	2209	50297-39-7 	Yes, SPET: 6000	No	Yes. The NOAEL of 760 mg/kg bw per day for the structurally related neohesperidin dihydrochalcone in a 90-day study in rats is 7600 times the estimated dietary exposure to No. 2209 when used as a flavouring agent.	Note 2	Neohesperidin dihydrochalcone 	No safety concern
(−)-Matairesinol	2210	580-72-3 	Yes, SPET: 7500	No	Yes. The NOAEL of 160 mg/kg bw per day for the structurally related 7-hydroxy-matairesinol in a 90-day study in rats is 1300 times the estimated dietary exposure to No. 2210 when used as a flavouring agent.	Note 2	7-Hydroxymatairesinol 	No safety concern

[a] Sixty-four flavouring agents in this group were previously evaluated by the Committee (Annex 1, references *149*, *202* and *211*).
[b] *Step 1*: One flavouring agent in this group (No. 2207) is in structural class II. Three flavouring agents in this group (Nos 2208–2210) are in structural class III.
[c] *Step 2*: All four 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 µg/day and 90 µg/day, respectively. All dietary exposure values are expressed in µg/day. The dietary exposure values listed represent the highest values calculated by either the SPET or the MSDI method. The SPET gave the highest estimated dietary exposure in each case.
[e] The MOEs were calculated based on the estimated dietary exposure calculated using the SPET.

Notes:

1. Phenolic glucosides are expected to undergo hydrolysis via epithelial cells or gut microflora to the corresponding phenol (aglycone).
2. Phenols are methylated or form sulfates or glucuronides prior to elimination in the urine or faeces.

Step 2. All four 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 for all four flavouring agents are above the thresholds of concern (i.e. 540 µg/day for class II, 90 µg/day for class III). Accordingly, the evaluation of all four flavouring agents proceeded to step A4.

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

Step A5. For myricitrin (No. 2207), the NOAEL of 884 mg/kg bw per day from a 52-week study in rats provides an adequate MOE of 18 000 in relation to the highest estimated dietary exposure (SPET = 3000 µg/day or 50 µg/kg bw per day) to No. 2207 when used as a flavouring agent. The Committee therefore concluded that myricitrin would not pose a safety concern at current estimated dietary exposures.

For naringin dihydrochalcone (No. 2208), the NOAEL of 500 mg/kg bw per day from short-term studies in rats provides an adequate MOE of 7500 in relation to the highest estimated dietary exposure (SPET = 4000 µg/day or 67 µg/kg bw per day) to No. 2208 when used as a flavouring agent. The Committee therefore concluded that naringin dihydrochalcone would not pose a safety concern at current estimated dietary exposures.

For 1-(2,4-dihydroxyphenyl)-3-(3-hydroxy-4-methoxyphenyl)propan-1-one (No. 2209), the NOAEL of 760 mg/kg bw per day for the structurally related substance neohesperidin dihydrochalcone from a 90-day study in rats provides an adequate MOE of 7600 in relation to the highest estimated dietary exposure (SPET = 6000 µg/day or 100 µg/kg bw per day) to No. 2209 when used as a flavouring agent. The Committee therefore concluded that 1-(2,4-dihydroxyphenyl)-3-(3-hydroxy-4-methoxyphenyl)propan-1-one would not pose a safety concern at current estimated dietary exposures.

For (–)-matairesinol (No. 2210), the NOAEL of 160 mg/kg bw per day for the structurally related 7-hydroxymatairesinol in a 90-day study in rats provides an adequate MOE of 1300 in relation to the highest estimated dietary exposure (SPET = 7500 µg/day or 125 µg/kg bw per day) to No. 2210 when used as a flavouring agent. The Committee therefore concluded that (–)-matairesinol would not pose a safety concern at current estimated dietary exposures.

Table 6 summarizes the evaluations of the four flavouring agents belonging to the phenol and phenol derivatives group (Nos 2207–2210).

Consideration of combined intakes from use as flavouring agents

The four flavouring agents in this phenol and phenol derivatives group have MSDI values of 0.03–182 µg/day. The Committee concluded that consideration of combined intakes is not necessary, because these flavouring agents would not contribute significantly to the combined intake of this group.

Conclusion

In the previous evaluations of flavouring agents in the phenol and phenol derivatives group, studies of acute toxicity, short-term and long-term toxicity (18 days to 2 years), carcinogenicity, genotoxicity and reproductive and developmental toxicity were available (Annex 1, references *150*, *203* and *212*).

For the present evaluation, biochemical data, acute, short-term and long-term studies of toxicity and genotoxicity studies were available for one flavouring agent in this group (No. 2207); biochemical data and short-term studies of toxicity were available for one flavouring agent in this group (No. 2208); biochemical data were available for one flavouring agent in this group (No. 2210); and genotoxicity data were available for one flavouring agent in this group (No. 2209). Genotoxicity and developmental toxicity studies were available for 7-hydroxymataresinol, a structurally related substance. The studies available for the present evaluation support the previous safety evaluations.

The Committee concluded that these four 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.

4.1.7 Phenyl-substituted aliphatic alcohols and related aldehydes and esters

Introduction

The Committee evaluated two additional flavouring agents belonging to the group of phenyl-substituted aliphatic alcohols and related aldehydes and esters. The additional flavouring agents included one ester (No. 2202) and one aldehyde (No. 2069), both containing phenyl substituents. Neither of these agents has previously been evaluated by the Committee.

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.

Ethyl 3-(2-hydroxyphenyl)propanoate (No. 2202) has been reported to occur in tonka beans.

Assessment of dietary exposure

The total annual volume of production of ethyl 3-(2-hydroxyphenyl)propanoate (No. 2202) is 0.1 kg in the USA, with no reported production volume for Europe or Japan. Dietary exposures were estimated using both the SPET and the MSDI method. The highest estimated dietary exposure for ethyl 3-(2-hydroxyphenyl)-propanoate (No. 2202) is 100 μg/day, the SPET value obtained from milk products (see Table 7).

The total annual volume of production of (±)-2-phenyl-4-methyl-2-hexenal (No. 2069) is 0.1 kg in Japan, with no reported production volume for the USA or Europe. The highest estimated dietary exposure for (±)-2-phenyl-4-methyl-2-hexenal (No. 2069) is 150 μg/day, the SPET value obtained from instant coffee and tea.

Absorption, distribution, metabolism and elimination

Information on the absorption, distribution, metabolism and elimination of flavouring agents belonging to the phenyl-substituted aliphatic alcohols and related aldehydes and esters group has previously been described in the monograph of the sixty-third meeting (Annex 1, reference *174*). Additional data on the in vitro metabolism of 2-methyl-3-(*p*-isopropylphenyl)propionaldehyde (No. 1465) and 2-phenylpropionaldehyde (No. 1467), flavouring agents structurally similar to No. 2202, were submitted that are in line with the data previously submitted.

Genotoxicity

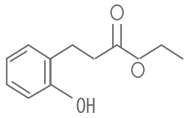
For the current evaluation, additional genotoxicity studies were available for 2-phenyl-2-butenal (No. 1474), a flavouring agent previously evaluated in this group.

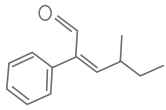
2-Phenyl-2-butenal (No. 1474) did not show mutagenic potential in bacterial reverse mutation assays in the absence or presence of metabolic activation. In an in vitro micronucleus test in cultured human lymphocytes, it showed genotoxic potential only in the absence of metabolic activation. Data from two in vivo micronucleus tests gave inconclusive results. The results of these in vivo studies would have been more convincing had direct evidence of systemic exposure to 2-phenyl-2-butenal been demonstrated. Also, as this substance was genotoxic only without metabolic activation in the in vitro micronucleus test, the Committee concluded that additional data are needed to address these concerns and conclude on the genotoxicity of 2-phenyl-2-butenal (No. 1474) and the other previously evaluated α,β-unsaturated 2-phenyl compounds in this group (Nos 1472, 1473 and 1476) and No. 2069.

The Committee concluded that the Procedure cannot be applied to No. 2069 until the concerns regarding genotoxicity are resolved.

Table 7

Summary of the results of the safety evaluations of phenyl-substituted aliphatic alcohols and related aldehydes 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					
Ethyl 3-(2-hydroxyphenyl)-propanoate	2202	20921-04-4 	No, SPET: 100	Notes 1 and 2	No safety concern

Flavouring agent	No.	CAS no. and structure	Conclusion
Flavouring agent not evaluated by the Procedure			
(±)-2-Phenyl-4-methyl-2-hexenal	2069	26643-92-5 	The Procedure cannot be applied to this substance until concerns regarding genotoxicity are addressed

[a] Twenty-two flavouring agents in this group were previously evaluated by the Committee (Annex 1, reference *173*).
[b] *Step 1*: Flavouring agent No. 2202 is in structural class I.
[c] *Step 2*: Flavouring agent No. 2202 can be expected to be metabolized to innocuous products.
[d] The threshold for human dietary exposure for structural class I is 1800 µg/day. The dietary exposure value is 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.

Notes:

1. Readily forms glucuronic acid conjugates, which are subsequently excreted in the urine.
2. Esters undergo rapid hydrolysis to liberate the corresponding alcohol and carboxylic acid.

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 agent in this group of phenyl-substituted aliphatic alcohols and related aldehydes and esters, the Committee assigned ethyl 3-(2-hydroxyphenyl)propanoate (No. 2202) to structural class I *(6)*.

Step 2. Ethyl 3-(2-hydroxyphenyl)propanoate (No. 2202) can be predicted to be metabolized to innocuous products. The evaluation of this flavouring agent therefore proceeded via the A-side of the Procedure.

Step A3. The highest estimated dietary exposure to ethyl 3-(2-hydroxyphenyl)propanoate (No. 2202) is below the threshold of concern

(i.e. 1800 μg/day for class I). The Committee therefore concluded that ethyl 3-(2-hydroxyphenyl)propanoate (No. 2202) would not pose a safety concern at current estimated dietary exposures.

Table 7 summarizes the evaluation of the additional flavouring agent belonging to the group of phenyl-substituted aliphatic alcohols and related aldehydes and esters (No. 2202).

Consideration of combined intakes from use as flavouring agents

The additional flavouring agent in this group of phenyl-substituted aliphatic alcohols and related aldehydes and esters that was evaluated according to the Procedure has a low MSDI value (0.01 μg/day). The Committee concluded that consideration of combined intakes is not necessary, because this additional flavouring agent would not contribute significantly to the combined intake of this flavouring group.

Conclusion

In the previous evaluation of flavouring agents in this group of phenyl-substituted aliphatic alcohols and related aldehydes and esters, biochemical studies and studies of acute toxicity, short-term toxicity and genotoxicity were available. The results of those studies did not raise safety concerns.

For the current evaluation, additional studies were available on flavouring agents previously evaluated in this group, including metabolism studies (Nos 1465 and 1467), short-term studies of toxicity (Nos 1465–1467), genotoxicity studies (No. 1474) and a study of reproductive toxicity (No. 1465).

The metabolism and toxicity data available for this evaluation generally supported those from the previous evaluation. However, the new genotoxicity studies on 2-phenyl-2-butenal (No. 1474) raise concerns regarding No. 1474 and the other previously evaluated α,β-unsaturated 2-phenyl compounds in this group (Nos 1472, 1473 and 1476) and No. 2069.

The Committee concluded that the Procedure cannot be applied to No. 2069 until the concerns regarding genotoxicity are resolved. The Committee recommended that the evaluations of the other α,β-unsaturated aldehydes in this group (Nos 1472–1474 and 1476) should be reconsidered at a future meeting, given the potential genotoxicity of 2-phenyl-2-butenal (No. 1474).

The Committee concluded that ethyl 3-(2-hydroxyphenyl)propanoate (No. 2202), which is an addition to the group of phenyl-substituted aliphatic alcohols and related aldehydes and esters evaluated previously, would not give rise to safety concerns at current estimated dietary exposures.

An addendum to the toxicological monograph was prepared.

4.1.8 Sulfur-containing heterocyclic compounds

Introduction

The Committee evaluated three flavouring agents belonging to the group of sulfur-containing heterocyclic compounds. The flavouring agents included one dithiazine (No. 2205), one thiazoline (No. 2206) and one thiophene (No. 1051). Two of the flavouring agents (Nos 2205 and 2206) have not been previously evaluated by the Committee, and their evaluations were conducted using the Procedure for the Safety Evaluation of Flavouring Agents (Annex 1, reference *131*). The third flavouring agent (No. 1051) was previously evaluated by the Committee at its fifty-ninth meeting (Annex 1, reference *161*) and was reconsidered by the current Committee because of concerns about potential mutagenicity. The Committee was informed that the flavouring industry is taking steps to remove this compound from the market.

The Committee previously evaluated 30 members of this group of flavouring agents at its fifty-ninth meeting (Annex 1, reference *161*). Based on the data available, the Committee concluded that all 30 members of this group of flavouring agents were of no safety concern at estimated dietary exposures. No. 1051 was a member of this group of flavouring agents.

The Committee also evaluated 17 additional members of this group of flavouring agents at its sixty-eighth meeting (Annex 1, reference *188*) and 12 additional members of this group of flavouring agents at its seventy-sixth meeting (Annex 1, reference *212*). The Committee concluded that all 29 additional flavouring agents were of no safety concern at estimated dietary exposures.

All three flavouring agents have been reported to occur naturally and can be found in shrimp, beef and sesame seed oil.

Assessment of dietary exposure

The annual volume of production of triethylthialdine (No. 2205) is 0.1 kg in Europe, and the annual volume of production of 2-isopropyl-4-methyl-3-thiazoline (No. 2206) is 0.1 kg in the USA.

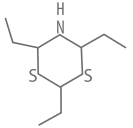
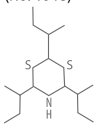
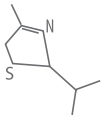
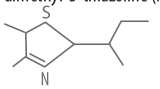
Dietary exposures were estimated for these two flavouring agents using the SPET and the MSDI method, with the highest values reported in Table 8. The estimated dietary exposures for Nos 2205 and 2206 range from 0.01 to 75 µg/day, with the SPET yielding the highest estimates in both cases.

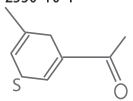
Absorption, distribution, metabolism and elimination

Information on the absorption, distribution, metabolism and elimination of the flavouring agents belonging to the group of sulfur-containing heterocyclic compounds has previously been described in the monographs of the fifty-ninth, sixty-eighth and seventy-sixth meetings (Annex 1, references *161*, *188*

Table 8

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?	*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							
Triethylthialdine	2205	54717-17-8 	No, SPET: 5	Yes. The NOAEL of 9.3 mg/kg bw per day for the structurally related 5,6-dihydro-2,4,6-tris-(2-methylpropyl)-4*H*-1,3,5-dithiazine (No. 1048) in a 90-day study in rats is 93 000 times the estimated dietary exposure to No. 2205 when used as a flavouring agent.	Notes 1 and 2	5,6-Dihydro-2,4,6-tris(2-methylpropyl)-4*H*-1,3,5-dithiazine (No. 1048) 	No safety concern
Structural class III							
2-Isopropyl-4-methyl-3-thiazoline	2206	67936-13-4 	No, SPET: 75	Yes. The NOAEL 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 1200 times the estimated dietary exposure to No. 2206 when used as a flavouring agent.	Notes 1 and 2	2-(2-Butyl)-4,5-dimethyl-3-thiazoline (No. 1059) 	No safety concern

Flavouring agent	No.	CAS no. and structure	Conclusion
Flavouring agent not evaluated according to the Procedure			
3-Acetyl-2,5-dimethylthiophene	1051	2530-10-1 	Inappropriate for use as a flavouring agent

[a] Fifty-nine flavouring agents in this group were previously evaluated by the Committee (Annex 1, references *161*, *188* and *212*). At the current meeting, No. 1051 was reconsidered in light of potential genotoxicity concerns.

[b] *Step 1*: One flavouring agent is in structural class II (No. 2205), and one flavouring agent is in structural class III (No. 2206).

[c] *Step 2*: The two flavouring agents in this group cannot be predicted to be metabolized to innocuous products.

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

[e] The MOEs were calculated based on the estimated dietary exposure calculated by the SPET.

Notes:

1. Flavin-dependent monooxygenase and cytochrome P450–dependent sulfoxidation and sulfone formation.
2. Cytochrome P450–dependent alkyl side-chain oxidation.

and *212*). Generally, dithiazine and thiazoline derivatives, being cyclic sulfides, are metabolized primarily by *S*-oxidation to yield the corresponding sulfoxides and sulfones. Other routes of metabolism for sulfur-containing heterocyclic compounds, including ring oxidation and cleavage, are also possible.

Genotoxicity

At the current meeting, the Committee re-evaluated No. 1051 due to concerns about potential mutagenicity. The Committee decided that the positive in vitro and in vivo mutagenicity data suggest a genotoxic risk to humans. Therefore, the Committee considered that it could not evaluate this flavouring agent according to the Procedure (see Table 8).

Application of the Procedure for the Safety Evaluation of Flavouring Agents

The evaluations for Nos 2205 and 2206 were conducted using the Procedure for the Safety Evaluation of Flavouring Agents, as described below.

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the above-mentioned flavouring agents, the Committee assigned flavouring agent No. 2205 to structural class II and flavouring agent No. 2206 to structural class III *(6)*.

Step 2. Neither of the flavouring agents in this group (Nos 2205 and 2206) 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 exposure for the flavouring agent in structural class II (No. 2205) is below the threshold of concern (i.e. 540 µg/day for class II). The highest estimated dietary exposure for the flavouring agent in structural class III (No. 2206) is also below the threshold of concern (i.e. 90 µg/day for class III). Accordingly, the evaluation of these flavouring agents proceeded to step B4.

Step B4. For triethylthialdine (No. 2205), the NOAEL of 9.3 mg/kg bw per day for the structurally related 5,6-dihydro-2,4,6-tris(2-methylpropyl)-4*H*-1,3,5-dithiazine (No. 1048) in a 90-day study in rats provides an MOE of 93 000 in relation to the highest estimated dietary exposure to No. 2205 (SPET = 5 µg/day or 0.1 µg/kg bw per day) when used as a flavouring agent. The Committee therefore concluded that triethylthialdine (No. 2205) would not pose a safety concern at current estimated dietary exposures.

For 2-isopropyl-4-methyl-3-thiazoline (No. 2206), the NOAEL of 1.2 mg/kg bw per day for the structurally related 2-(2-butyl)-4,5-dimethyl-3-thiazoline (No. 1059) in a 90-day study in rats provides an MOE of 1200 in relation to the highest estimated dietary exposure to No. 2206 (SPET = 75 µg/day or 1 µg/kg bw per day) when used as a flavouring agent. The Committee therefore concluded

that 2-isopropyl-4-methyl-3-thiazoline (No. 2206) would not pose a safety concern at current estimated dietary exposures.

Table 8 summarizes the evaluations of the two additional flavouring agents belonging to the group of sulfur-containing heterocyclic compounds (Nos 2205 and 2206).

Consideration of combined intakes from use as flavouring agents

The two additional flavouring agents in this group of sulfur-containing heterocyclic compounds have low MSDI values (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.

Conclusion

In the previous evaluations of flavouring agents in this group of sulfur-containing heterocyclic compounds, studies of acute toxicity, short-term toxicity and genotoxicity were available (Annex 1, references *161*, *188* and *212*). None of the flavouring agents in this group raised safety concerns in the previous evaluations.

For the present evaluation, additional short-term studies of toxicity were available for two flavouring agents previously evaluated in this group (Nos 1048 and 2106); studies of in vitro genotoxicity were available for six flavouring agents previously evaluated in this group (Nos 1038, 1045, 1050, 1051, 1059 and 1759), and studies of in vivo genotoxicity were available for two flavouring agents previously evaluated in this group (Nos 1050 and 1051). For one compound (No. 1051) in this group, recently conducted mutagenicity studies suggest a potential mutagenic risk of the substance itself or a reactive metabolite.

The Committee concluded that the new data available indicate that 3-acetyl-2,5-dimethylthiophene (No. 1051) is mutagenic in vitro and in vivo; although the mechanism of mutagenesis is unknown, the possibility of a mutagenic response in humans cannot be discounted. Additional toxicity and metabolic studies that would have been relevant in assessing the biological significance of the mutagenicity evidence reported in in vitro and in vivo assays were not available. The Committee considered it inappropriate for such a compound to be used as a flavouring agent or for any other food additive purpose and withdrew the previous conclusion of the Committee. The Committee is also aware that the flavouring industry has already taken steps to remove this compound from the market.

The Committee concluded that the flavouring agents Nos 2205 and 2206, which are additions to the group of sulfur-containing heterocyclic compounds evaluated previously, would not give rise to safety concerns at current estimated dietary exposures.

An addendum to the toxicological monograph was prepared.

4.2 Specifications of identity and purity of flavouring agents

The Committee received information related to specifications for the 26 new flavouring agents from the call for data for the present meeting. At the current meeting, no specifications were prepared for α-ionene (No. 2193) because it was not evaluated toxicologically (see section 4.1.1) or for (±)-2-phenyl-4-methyl-2-hexenal (No. 2069) due to unresolved toxicological concerns (see section 4.1.7). Specifications were prepared for 24 flavouring agents. In addition to the free acid form of No. 2204, the Committee was notified that the hemisulfate monohydrate salt of this flavouring was also used in commerce. As a result, specifications were also prepared for the hemisulfate monohydrate salt of No. 2204, and this substance was identified as No. 2204.1.

Specifications established at the seventy-sixth meeting in 2012 (Annex 1, reference *211*) for nerolidol oxide (No. 2137) were maintained.

Specifications established at the fifty-ninth meeting in 2002 (Annex 1, reference *160*) for 3-acetyl-2,5-dimethylthiophene (No. 1051) were withdrawn based on toxicological concerns (see section 4.1.8).

5. Future work and recommendations

General considerations

Threshold of toxicological concern (TTC) principle: update on a WHO project and implications for the Procedure for the Safety Evaluation of Flavouring Agents

The Committee recommended that a proposal regarding a revised JECFA decision-tree for the evaluation of flavours based on application of the TTC principle in the risk assessment of chemicals should be further considered and a proposal prepared for consideration at the next JECFA meeting at which flavours will be evaluated.

Need for an approach for prioritizing flavouring agents for re-evaluation

The Committee held a preliminary discussion concerning the fact that the submission of additional toxicology data, including genotoxicity data, and/or exposure data for new or previously evaluated flavouring agents may trigger the need for re-evaluation of previously evaluated flavouring agents. The Committee recommended that an approach be developed for prioritizing flavouring agents for re-evaluation based on all available toxicological data and updated exposure estimates.

Limits for lead in specifications of food additives for use in infant formulas

The Committee referred back to CCFA on whether specific purity criteria for additives for use in infant formulas should be considered and appropriate ways to present these criteria (e.g. establishing specifications for additives for use in infant formulas only; establishing different purity limits for additives for use in infant formulas in existing specifications).

Specific food additives (other than flavouring agents)

Citric and fatty acid esters of glycerol (CITREM)

The Committee noted that the test method for the determination of total citric acid in the specifications monograph for CITREM currently employs a gas chromatographic method using a packed column. The Committee recommended the submission of data for a suitable method using a capillary/wide-bore column to replace the current method for consideration at a future meeting.

Gardenia yellow

The Committee noted that it is not clear whether the material tested toxicologically was representative of gardenia yellow. In addition, the available toxicity studies have

not been conducted following internationally recognized guidelines, and a number of studies were performed using non-relevant routes of administration. Finally, there are no long-term toxicity, carcinogenicity, reproductive toxicity or developmental toxicity studies available.

In order to establish specifications, the Committee requires:

- information on the manufacturing process, including purification steps;
- analytical data on the composition of the substance, including the total amount of colouring matter and relevant compounds of known biological activity, such as geniposide and genipin;
- data on loss on drying;
- information on a method of assay;
- analytical data on at least five different batches of commercial materials supporting the specifications; and
- data on stability in food.

Lutein esters from *Tagetes erecta*

New tentative specifications were prepared. The Committee requested the following information, **by the end of 2015**, to complete the safety assessment:

- details on the manufacturing process, including purification steps;
- detailed analytical data on the full composition of at least five different batches of commercially available product to support the specifications;
- method of analysis to determine carotenoid composition; and
- method of analysis to determine the composition of the non-carotenoid lipidic fraction.

Octenyl succinic acid (OSA)–modified gum arabic

The existing specifications were revised and their tentative status was maintained, pending the submission of the following information, **by the end of 2015**:

- data on the manufacturing process, including purification steps;
- chemical characterization of the product in commerce;
- updated analytical methods for the determination of esterified (bound) and residual (free) OSA;
- results of the analysis of at least five batches of product in commerce; and
- applicability of the high-performance liquid chromatographic method for the determination of residual OSA.

Modified starches

The existing specifications monograph for modified starches includes 16 different modified starches, which complicates revisions of the specifications for any individual modified starch. Therefore, the Committee recommended that the specifications monograph for the modified starches be split into 16 individual specifications monographs.

The Committee, as noted at its seventy-sixth meeting, considered that it would also be necessary to revise the specifications for all the modified starches, including test methods, at future meetings.

Pectin

The Committee requested additional data to support the safety evaluation of pectin in infant formula, including an explanation for the decreased feed intake and body weight gain in neonatal pigs.

Flavouring agents

Phenyl-substituted aliphatic alcohols and related aldehydes and esters

The Committee concluded that the Procedure for the Safety Evaluation of Flavouring Agents could not be applied to (±)-2-phenyl-4-methyl-2-hexenal (No. 2069) until concerns regarding genotoxicity are resolved. In addition, the evaluations of the other α,β-unsaturated aldehydes in this group (Nos 1472–1494 and 1476) should be reconsidered at a future meeting, given the potential genotoxicity of 2-phenyl-2-butenal (No. 1474).

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

Additional toxicological and/or dietary exposure information is required to complete the toxicological evaluation of one flavouring agent (No. 2188). The Committee was aware of additional genotoxicity data reporting equivocal results for a structurally related compound; therefore, information to address any concerns regarding potential genotoxicity should also be provided.

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Annex 1

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

1. *General principles governing the use of food additives* (First report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 15, 1957; WHO Technical Report Series, No. 129, 1957 (out of print).

2. *Procedures for the testing of intentional food additives to establish their safety for use* (Second report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 17, 1958; WHO Technical Report Series, No. 144, 1958 (out of print).

3. *Specifications for identity and purity of food additives (antimicrobial preservatives and antioxidants)* (Third report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as *Specifications for identity and purity of food additives,* Vol. I. *Antimicrobial preservatives and antioxidants*, Rome, Food and Agriculture Organization of the United Nations, 1962 (out of print).

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).

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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).

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11. *Specifications for the identity and purity of food additives and their toxicological evaluation: some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases* (Ninth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings 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.

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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.

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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.

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Annex 2

Toxicological information and information on specifications

Food additives considered for specifications only

Food additive	Specifications
Citric acid	R[a]
Gellan gum	R[b]
Polyoxyethylene (20) sorbitan monostearate	R[c]
Potassium aluminium silicate	R[d]
Quillaia extract (Type 2)	R[e]

R: existing specifications revised

[a] The method for the oxalate limit test was amended.

[b] The method of assay in the specifications refers to the alginates assay method. This method was replaced by a method without the use of mercury.

[c] Criteria for saponification and hydroxyl values were revised.

[d] The Committee reviewed the existing data as well as new information received from the sponsor and noted that potassium aluminium silicate (PAS) stabilizes the formed layers of titanium dioxide and/or iron oxide in the PAS-based pearlescent pigments. Therefore, the Committee concluded that PAS exerts a technological effect in the PAS-based pearlescent pigments; as a result, PAS could not be considered to function as a carrier according to the Codex definition for carrier. Hence, the Committee decided to delete the functional use as carrier in the specifications.

[e] The upper limit in the loss on drying specification was increased from 80% to 90%.

Food additives evaluated toxicologically and assessed for dietary exposure

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological or safety recommendations
Benzoe tonkinensis	R[a]	Given the no-observed-adverse-effect level (NOAEL) of 500 mg/kg body weight (bw) per day for Benzoe tonkinensis identified in a 90-day oral toxicity study in rats and the previously established ADIs for the major components of Benzoe tonkinensis (benzoic acid, benzyl benzoate and vanillin), **the Committee confirmed the conclusion from the seventy-fourth meeting that Benzoe tonkinensis would not be of safety concern at current estimated dietary exposures**, provided that it complies with the specifications prepared at the current meeting, when used as a flavouring agent and in accordance with good manufacturing practice.
Carrageenan (for use in infant formula and formula for special medical purposes intended for infants)	R	The margins of exposure (MOEs) between the NOAEL of 430 mg/kg bw per day (2250 mg/kg formula), the highest dose tested, from a neonatal pig study and human infant exposures at 2–4 weeks of age range from 2 to 12 on a body weight basis and from 2 to 8 on a concentration basis. The Committee noted that although the MOEs are small in magnitude, they are derived from a neonatal pig study in which the highest dose tested was without adverse effects on the gut or on immune parameters, supported by a neonatal minipig study. These new studies allay the earlier concerns that carrageenan, which is unlikely to be absorbed, may have a direct effect on the immature gut. The Committee also took account of the previous toxicological database on carrageenan, which did not indicate other toxicological concerns. It also noted that at carrageenan concentrations higher than 2500 mg/kg, formula becomes highly viscous, which adversely affects palatability and growth.

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological or safety recommendations
		The Committee concluded that the use of carrageenan in infant formula or formula for special medical purposes at concentrations up to 1000 mg/L is not of concern. The Committee recognized that there is variability in medical conditions among infants requiring formulas for special medical purposes that contain the higher levels of carrageenan, and the Committee noted that these infants would normally be under medical supervision.
Citric and fatty acid esters of glycerol (CITREM) (for use in infant formula and formula for special medical purposes intended for infants)	R	The Committee considered it unlikely that consumption of formulas containing typical levels of CITREM used in powdered formulas (up to 2.7 g/L as reconstituted), which is equivalent to an exposure to citrate of 440 mg/kg bw per day for the very young infant at the 95th percentile energy intake, would cause diarrhoea. At the high end of the requested range for use (up to 9 g/L), which is equivalent to an exposure to citrate of 1140 mg/kg bw per day for the very young infant at the 95th percentile energy intake, diarrhoea might occur in some infants. **The Committee concluded that there are no toxicological concerns about the use of CITREM in infant formula and formula for special medical purposes at concentrations up to 9 g/L.** At the higher use levels, there is a possibility of diarrhoea from free citric acid released from formula containing CITREM. Given the paucity of clinical data and the fact that exposure assumptions for citric acid have been maximized, it is difficult to estimate the risk of diarrhoea, but it is considered to be low.
Gardenia yellow	No[b]	Given the deficiencies in the toxicological and specifications databases, including incomplete data on the manufacturing process and composition of the material, missing toxicological studies (e.g. long-term toxicity, carcinogenicity, reproductive toxicity and developmental toxicity), the inadequate characterization of the test material and limited reporting of the available studies, **the Committee was unable to evaluate gardenia yellow proposed for use as a food colour.**
Lutein esters from *Tagetes erecta*	N, T[c]	The Committee concluded that there was no need to establish a numerical ADI. This decision was based on a number of factors, including the absence of any observed toxicity of lutein or lutein esters in any of the available toxicological studies in animals; the absence of any adverse effects in humans consuming lutein or lutein esters; the large MOE (>1500) between the NOAEL for lutein in a new 13-week study in rats and the estimated dietary exposure of 0.32 mg/kg bw per day (from additive and natural sources); a 2-fold increase in the NOAEL for lutein as a result of another new 13-week study; and the fact that lutein esters from *Tagetes erecta* are considered to be substitutional for other lutein extracts. **The Committee established a temporary ADI "not specified"[e] for lutein esters from *Tagetes erecta*.** The ADI was made temporary because the specifications for lutein esters from *Tagetes erecta* were tentative. The Committee considered establishing a group ADI "not specified" for lutein esters from *Tagetes erecta* that would include lutein from *Tagetes erecta* and synthetic zeaxanthin and related xanthophylls, but this would be possible only when the specifications for lutein esters from *Tagetes erecta* are finalized.

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological or safety recommendations
Octenyl succinic acid (OSA)–modified gum arabic	R, T[c]	The tentative status of the specifications was maintained pending the submission of additional data. The Committee noted that additional safety data may also be needed to complete the evaluation of OSA-modified gum arabic. **The Committee decided that the temporary ADI "not specified" will be withdrawn unless adequate data to complete the safety evaluation are submitted by the end of 2015.**
Octenyl succinic acid (OSA)–modified starch (starch sodium octenyl succinate) (for use in infant formula and formula for special medical purposes intended for infants)	R[d]	Taking into account the overall low toxicity of OSA-modified starch, the conservatism in the NOAEL, which was the highest dose tested in a study in neonatal animals, and in the exposure assessments, as well as the supporting evidence from clinical trials and post-marketing surveillance, **the Committee concluded that the consumption of OSA-modified starch in infant formula or formula for special medical purposes intended for infants is not of concern at use levels up to 20 g/L.** New data available since the twenty-sixth meeting confirm the very low toxicity of OSA-modified starch, and **the Committee confirmed the ADI "not specified" established at that meeting for its use as a food additive for the general population.**
Paprika extract	M	**The Committee established an ADI for paprika extract used as a food colour of 0–1.5[f] mg/kg bw, expressed as total carotenoids**, with the application of an uncertainty factor of 100 to the NOAEL of 153 mg/kg bw per day from a 2-year toxicity and carcinogenicity study in rats. The Committee concluded that dietary exposure to paprika extract used as a food colour does not present a health concern.
Pectin (for use in infant formula and formula for special medical purposes intended for infants)	M	In a 3-week study in neonatal pigs fed pectin-containing milk replacer, the NOAEL was 847 mg/kg bw per day, with decreased feed intake and body weight gain observed at 3013 mg/kg bw per day. Using the NOAEL from this study, the MOEs were estimated to be 0.9 for infants with median energy intake and 0.8 for infants with high (95th percentile) energy intake. The Committee concluded that estimated exposure to pectin from its use in infant formula is in the region of the NOAEL derived from the neonatal pig study and close to the LOAEL based on decreased feed intake and body weight gain. While no overt toxicological effects were observed in the neonatal pigs, decreased food intake and body weight gain would be considered an undesirable effect in human infants. The available clinical studies were mainly conducted with pectin or pectin-derived oligosaccharides at concentrations of 0.2% or less and therefore do not provide support for tolerance and normal growth at the proposed use level. Therefore, **the Committee concluded that the use of pectin in infant formulas at the maximum proposed use level (0.5%) is of concern.**

M: existing specifications maintained; N: new specifications; No: no specifications prepared; R: existing specifications revised; T: tentative specifications

[a] The tentative qualification of the specifications was removed.

[b] No specifications were prepared. Information is required to prepare specifications.

[c] Additional information is required to finalize the specifications (see section 5).

[d] The analytical method for the determination of the octenyl succinyl group in starch sodium octenyl succinate was amended.

[e] 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.

[f] The Committee noted that although derived values, such as health-based guidance values, should be rounded to a single significant figure, it decided to use two significant figures in the present case, as the impact of rounding to one significant figure would be more than 30%.

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

A. Aliphatic and alicyclic hydrocarbons

The Committee determined that the flavouring agent α-ionene (No. 2193), which was submitted for evaluation as part of this flavouring agent group, did not fit into this group on the basis of its chemical structure and did not evaluate α-ionene.

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class I			
1-Octene	2191	N	No safety concern
2,4-Nonadiene	2192	N	No safety concern
4-Methyl-*cis*-2-pentene	2194	N	No safety concern
1-Nonene	2195	N	No safety concern
1,3,5,7-Undecatetraene	2196	N	No safety concern
Mixture of methyl cyclohexadiene and methylene cyclohexene	2197	N	No safety concern

N: new specifications

B. Aliphatic and aromatic ethers

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class III			
Cassyrane	2189	N	No safety concern
1-Cyclopropanemethyl-4-methoxybenzene	2190	N	No safety concern
Nerolidol oxide	2137	M	No safety concern

M: existing specifications maintained; N: new specifications

C. Ionones and structurally related substances

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class I			
β-Isomethylionone	2186	N	No safety concern
Pseudoionone	2187	N	No safety concern
trans-α-Damascone	2188	N	Additional data required to complete evaluation

N: new specifications

D. Miscellaneous nitrogen-containing substances

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class III			
3-[3-(2-Isopropyl-5-methylcyclohexyl)-ureido]-butyric acid ethyl ester	2203	N	No safety concern
4-Amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid (and its hemisulfate monohydrate salt)	2204 2204.1	N	No safety concern

N: new specifications

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

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class II			
2,2,6,7-Tetramethylbicyclo[4.3.0]nona-4,9(1)-dien-8-ol	2198	N	No safety concern
dl-Camphor	2199	N	No safety concern
l-Fenchone	2200	N	No safety concern
2,2,6,7-Tetramethylbicyclo[4.3.0]nona-4,9(1)-dien-8-one	2201	N	No safety concern

N: new specifications

F. Phenol and phenol derivatives

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class II			
Myricitrin	2207	N	No safety concern
Structural class III			
Naringin dihydrochalcone	2208	N	No safety concern
1-(2,4-Dihydroxyphenyl)-3-(3-hydroxy-4-methoxyphenyl)propan-1-one	2209	N	No safety concern
(−)-Matairesinol	2210	N	No safety concern

N: new specifications

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

The Committee concluded that the Procedure could not be applied to (±)-2-phenyl-4-methyl-2-hexenal (No. 2069) until concerns regarding genotoxicity are resolved. In addition, the evaluations of the other α,β-unsaturated aldehydes in this group (Nos 1472–1494 and 1476) should be reconsidered at a future meeting, given the potential genotoxicity of 2-phenyl-2-butenal (No. 1474).

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class I			
Ethyl 3-(2-hydroxyphenyl)propanoate	2202	N	No safety concern

N: new specifications

H. Sulfur-containing heterocyclic compounds

The Committee concluded that 2,5-dimethyl-3-acetylthiophene (No. 1051) is mutagenic in vitro and in vivo and considered that it is inappropriate for such a compound to be used as a flavouring agent or for any other food additive purpose. It therefore withdrew the previous conclusion of the Committee. The Committee is also aware that the flavouring industry has already taken steps to remove this compound from the market. Specifications established at the fifty-ninth meeting for No. 1051 were also withdrawn based on toxicological concerns.

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class II			
Triethylthialdine	2205	N	No safety concern
Structural class III			
2-Isopropyl-4-methyl-3-thiazoline	2206	N	No safety concern

N: new specifications

Annex 3

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

JECFA No.	Flavouring agent	Minimum assay value	Secondary components	Comments on secondary components
Aliphatic and alicyclic hydrocarbons				
2192	2,4-Nonadiene	79–80%	10–11% 1,3-nonadiene; 9–10% other nonadiene isomers	1,3-Nonadiene and other nonadiene isomers are anticipated to undergo oxidative metabolism to the corresponding epoxide followed by hydrolysis by epoxide hydrolase and glucuronic acid conjugation and elimination in the urine

Annex 4

Meeting agenda

Food and Agriculture Organization of the United Nations

World Health Organization

79th JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES (JECFA)
WHO Headquarters, Geneva, 17–26 June 2014

Opening:
Salle B, 17 June 2014, 9.30h

Agenda

1. Opening

2. Election of Chairperson and Vice-Chairperson, appointment of Rapporteurs

3. Adoption of Agenda

4. Declarations of Interests (information by the Secretariat on any declared interests and discussion)

5. Matters of interest arising from previous Sessions of the Codex Committee on Food Additives
   a. Report from CCFA - questions for action

6. Critical issues and questions from Working Papers (first brief round of discussion on all subjects to inform the full committee)

7. Evaluations

   Food Additives

   7.1 Toxicological evaluation, exposure assessment, and establishment of specifications:
   - Carrageenan (INS 407)
   - Citric acid esters of mono- and diglycerides of fatty acids (CITREM) (INS 472c)
   - Gardenia yellow (crocin)
   - OSA-modified starch (starch sodium octenyl succinate) (INS 1450)
   - OSA-modified gum arabic

- Paprika extract
- Pectin (INS 440)
- *Tagetes* extract (INS 161b(ii))

7.2 Food additives for revision of specifications only:
- Benzoe tonkinesis
- Citric acid
- Gellan gum (INS 418)
- Polyoxyethylene (20) sorbitan monostearate (polysorbate 60) (INS 435)
- *Quillaia* extract, type 2 (INS 999(ii))

Flavourings

7.3 New compounds as additions to previously evaluated groups of related flavouring substances:
- Ionones and structurally related substances
- Aliphatic and aromatic ethers
- Aliphatic and alicyclic hydrocarbons
- Monocyclic and bicyclic secondary alcohols, ketones, and related esters
- Phenyl-substituted aliphatic alcohols and related aldehydes and esters
- Miscellaneous nitrogen-containing substances
- Sulfur-containing heterocyclic compounds
- Phenol and phenol derivatives

8. Other matters to be considered (general considerations):
   a. For information:
      i. Update on GEMS/Food cluster diets
      ii. FOSCOLLAB
      iii. New FOS website
   b. For discussion:
      i. Threshold of toxicological concern principle: update on WHO project and implication for decision-tree for the evaluation of flavours
      ii. Limits for lead in specifications of food additives for infant foods

9. Other matters as may be brought forth by the Committee during discussions at the meeting.

10. Adoption of the report.

SELECTED WHO PUBLICATIONS OF RELATED INTEREST

Evaluation of Certain Veterinary Drug Residues in Food
Seventy-eighth Report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 988, 2014 (127 pages)

Toxicological Evaluation of Certain Veterinary Drug Residues in Food
Seventy-eighth Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)
WHO Food Additives Series, No. 69, 2014 (241 pages)

Evaluation of Certain Food Additives and Contaminants
Seventy-seventh Report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 983, 2013 (75 pages)

Safety Evaluation of Certain Food Additives and Contaminants
Seventy-seventh Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)
WHO Food Additives Series, No. 68, 2013 (335 pages)

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 (190 pages)

Safety Evaluation of Certain Food Additives
Seventy-sixth Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)
WHO Food Additives Series, No. 67, 2012 (335 pages)

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 (108 pages)

Toxicological Evaluation of Certain Veterinary Drug Residues in Food
Seventy-fifth Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)
WHO Food Additives Series, No. 66, 2012 (183 pages)

Safety Evaluation of Certain Food Additives and Contaminants
Seventy-fourth Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)
WHO Food Additives Series, No. 65, 2012 (825 pages)

Further information on these and other WHO publications can be obtained from
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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, and to prepare 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 eight food additives (Benzoe tonkinensis; carrageenan; citric and fatty acid esters of glycerol; gardenia yellow; lutein esters from *Tagetes erecta*; octenyl succinic acid–modified gum arabic; octenyl succinic acid–modified starch; paprika extract; and pectin) and eight groups of flavouring agents (aliphatic and alicyclic hydrocarbons; aliphatic and aromatic ethers; ionones and structurally related substances; miscellaneous nitrogen-containing substances; monocyclic and bicyclic secondary alcohols, ketones and related esters; phenol and phenol derivatives; phenyl-substituted aliphatic alcohols and related aldehydes and esters; and sulfur-containing heterocyclic compounds).

Specifications for the following food additives were revised: citric acid; gellan gum; polyoxyethylene (20) sorbitan monostearate; potassium aluminium silicate; and *Quillaia* extract (Type 2).

Annexed to the report are tables summarizing the Committee's recommendations for dietary exposures to and toxicological evaluations of all of the food additives and flavouring agents considered at this meeting.

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