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1000

Evaluation of certain food additives

Eighty-second report of the Joint FAO/WHO Expert Committee on Food Additives

Food and Agriculture Organization of the United Nations

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List of abbreviations

ADI	acceptable daily intake
AUC	area under the concentration–time curve
bw	body weight
CAS	Chemical Abstracts Service
CCCF	Codex Committee on Contaminants in Foods
CCFA	Codex Committee on Food Additives
CCFA48	Forty-eighth Session of the Codex Committee on Food Additives
CCFA49	Forty-ninth Session of the Codex Committee on Food Additives
CCFO	Codex Committee on Fats and Oils
CCNFSDU	Codex Committee on Nutrition and Foods for Special Dietary Uses
CITREM	citric and fatty acid esters of glycerol
C_{max}	maximum concentration
CYP	cytochrome P450
DNA	deoxyribonucleic acid
EFSA	European Food Safety Authority
EINECS	European Inventory of Existing Commercial Chemical Substances
EU	European Union
EU-16	Austria, Belgium, Czech Republic, Denmark, Finland, France, Greece, Ireland, Italy, Luxembourg, Netherlands, Portugal, Romania, Spain, Sweden, United Kingdom
F	filial generation (e.g. F_1, F_2, F_3)
FAO	Food and Agriculture Organization of the United Nations
FSANZ	Food Standards Australia New Zealand
GC-FID	gas chromatography–flame ionization detection
GC-MS	gas chromatography–mass spectrometry
GRAS	Generally Recognized As Safe
GSFA	(Codex) General Standard for Food Additives
GST	glutathione *S*-transferase
HPLC	high-performance liquid chromatography
ICP-AES	inductively coupled plasma atomic emission spectrometry
ICP-MS	inductively coupled plasma mass spectrometry
ICP-OES	inductively coupled plasma optical emission spectrometry
Ig	immunoglobulin (e.g. IgA, IgG, IgM)
INS	International Numbering System for Food Additives
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level
ML	maximum level
MOE	margin of exposure

mRNA	messenger ribonucleic acid
MSDI	maximized survey-derived intake
NHANES	National Health and Nutrition Examination Survey (USA)
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
OECD	Organisation for Economic Co-operation and Development
OSA	octenyl succinic acid
pAOS	pectin-derived acidic oligosaccharides
PEG	polyethylene glycol
PVA	polyvinyl alcohol
S9	9000 × *g* supernatant fraction from liver homogenate
SDG	Sustainable Development Goal
SPET	single-portion exposure technique
TLR	Toll-like receptor
T_{max}	time to reach the maximum concentration
TTC	threshold of toxicological concern
UN	United Nations
USA	United States of America
USFDA	United States Food and Drug Administration
WHO	World Health Organization
w/w	weight per weight

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. 73, in preparation.

Specifications are issued separately by FAO under the title:

Compendium of food additive specifications. FAO JECFA Monographs 19, 2016.

1. Introduction

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) met in Geneva from 7 to 16 June 2016. 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 noted that this year marks the sixtieth anniversary of JECFA, which is one of the longest standing expert committees at WHO and one of the best examples of collaboration between two agencies of the United Nations (UN). Providing scientific advice to Member States and to the Codex Alimentarius Commission is at the heart of the mandate of JECFA.

Dr Miyagishima informed participants that JECFA was born out of a recommendation from the Joint FAO/WHO Conference on Food Additives, held in Geneva in September 1955. JECFA first met in December 1956 in Rome, a meeting that was attended by eight members and three observers. Its work initially was to evaluate the safety of food additives and was later extended to contaminants, naturally occurring toxicants and residues of veterinary drugs in food.

Sixty years of JECFA also brings challenges. Member States now expect to receive scientific advice "for free", and the parent organizations, especially WHO, are facing difficulty in sustaining funding to JECFA and other expert bodies. WHO and FAO will continue to advocate the services that JECFA is delivering to Member States and to strengthen the Secretariat to better serve the experts.

In 2015, the UN adopted Agenda 2030, with its 17 Sustainable Development Goals (SDGs). Food safety is an important element in SDG 2 (End hunger, achieve food security and improved nutrition and promote sustainable agriculture) and SDG 3 (Ensure healthy lives and promote well-being for all at all ages), but food safety is more important than ever in many other SDGs for achieving sustainable growth in health and well-being. This year, 2016, also coincides with the beginning of the UN Decade of Action for Nutrition.

This meeting is dedicated to the evaluation of food additives, including flavouring agents. One of the tasks before this Committee is to re-evaluate the safety of several widely used food colours. The Committee will also continue to work on updating the methods and principles for health risk assessment of food additives, including a dedicated discussion on the Procedure for the Safety Evaluation of Flavouring Agents.

Dr Miyagishima noted that experts, whether from academia or from national agencies, are the critical asset of JECFA and ensure its neutrality and excellence. He 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 noted that the process by which FAO and WHO generate scientific advice is under global scrutiny, and, if an unfounded allegation is directed to its expert bodies and experts, WHO and FAO will do their best to defend their reputations. Dr Miyagishima closed by expressing his sincere gratitude to participants for providing their time and expertise to this important work and to their organizations for agreeing to put their experts at the disposal of JECFA.

1.1 Declarations of interests

The Secretariat informed the Committee that all experts participating in the eighty-second meeting had completed declaration of interest forms. The following declared interests and potential conflicts were discussed by the Committee. Dr Josef Schlatter holds investments in a flavouring company, and he was excluded from discussions on flavouring agents. Professor Glenn Sipes participated on a Generally Recognized As Safe (GRAS) panel on steviol glycosides and was excluded from discussions on these compounds. He also chairs a safety expert panel for the Research Institute for Fragrance Materials. Professor Gary Williams provides genotoxicity testing services on fragrances. Because of the potential overlap between fragrances and flavouring agents, Professors Sipes and Williams did not participate in the decision-making process on flavouring agents. No other conflicts of interest were identified.

1.2 Modification of the agenda

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

- Only very limited data were received on *Acacia polyacantha* var. *campylacantha*, kakamut gum, arabino-galactan protein complex, which do not allow an assessment. It was therefore removed from agenda item 7.1 (Toxicological evaluation, exposure assessment and establishment of specifications).
- Aspartame (International Numbering System [INS] No. 951) was added to agenda item 7.2 (Food additives for revision of specifications and analytical methods).
- Lutein esters from *Tagetes erecta* was on the agenda for the revision of specifications (agenda item 7.2). As the acceptable daily intake (ADI) had been made temporary because the specifications were tentative, lutein esters from *Tagetes erecta* was moved to agenda item 7.1.

- Sodium dihydrogen phosphate (INS No. 339(i)) was withdrawn by the sponsor and was removed from agenda item 7.2.
- The flavouring agent beta-angelicalactone (No. 2222) in the group Aliphatic, alicyclic-fused and aromatic-fused ring lactones was withdrawn by the sponsor and was removed from agenda item 7.3 (Toxicological evaluation, exposure assessment and establishment of specifications for certain flavourings).

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 81 previous meetings of the Committee (Annex 1). The present meeting was convened on the basis of a recommendation made at the seventy-ninth meeting (Annex 1, reference *220*).

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) (sections 3 and 4 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-eighth 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 eightieth meeting of JECFA (Annex 1, reference *223*).

The Forty-eighth Session of CCFA (CCFA48) *(2)* noted the conclusions of the eightieth meeting of JECFA on the safety of nine substances. CCFA48 agreed to revise the maximum level (ML) for benzoates in food category 14.1.4 to 250 mg/kg with Note 13 "as benzoic acid", to revise Note 301 to read "interim maximum level until CCFA49" and to delete Note 123. Lipase from *Fusarium heterosporum* expressed in *Ogataea polymorpha* (INS No. 1104) and maltotetraohydrolase from *Pseudomonas stutzeri* expressed in *Bacillus licheniformis* will be included in the database on processing aids (http://www.ccfa.cc/IPA/). For magnesium stearate (INS No. 470(iii)), CCFA48 recommended the adoption of the provisions in Table 3 of the Codex General Standard for Food Additives (GSFA) *(3)*.

Work on more than 400 provisions of the GSFA was finalized, and the adoption of specifications for the identity and purity of eight substances (four new specifications and four revised specifications) prepared by the eightieth meeting of JECFA and the revocation of the specifications for aluminium silicate (INS No. 559) and calcium aluminium silicate (INS No. 556) were recommended. CCFA48 also assigned new INS numbers to five food additives and amended the technological purpose for "Emulsifying salt" and "Stabilizer" as well as the name for INS No. 1101(i) and functional classes and technological purposes for the additive polyvinyl alcohol (PVA)–polyethylene glycol (PEG) graft copolymer (INS No. 1209).

CCFA48 agreed on a revised priority list of substances for evaluation (or re-evaluation) by JECFA, which includes 39 substances and 83 flavouring agents. With respect to two of these priority substances, it was understood that commitment for the submission of full dossiers (data and sponsor) for sodium sorbate (INS No. 221) would be confirmed no later than CCFA49, whereas confirmation of technological justification from the Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU) for gellan gum (INS No. 418) is pending. It was agreed that CCNFSDU needed to confirm the technological need for food additives intended for use in infant formula prior to their inclusion in the CCFA priority list. It was also agreed to request that China and the USA consider aspects related to the prioritization of substances for JECFA evaluation in the discussion paper on CCFA work management.

CCFA48 continued work on the alignment of food additive provisions in the Codex standards and the corresponding provisions of the GSFA. Information on use levels for adipic acid (INS No. 355) in various food categories will be provided to the JECFA Secretariat for exposure assessment. It was agreed to continue with the current practice of addressing the use of secondary additives by using notes within the current GSFA food category system. The revision of sections 4.1(c) and 5.1(c) of the Codex General Standard for the Labelling of Food Additives When Sold As Such *(4)* to align the terminology related to flavourings was agreed for adoption by the Thirty-ninth Session of the Codex Alimentarius Commission.

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)*.

2.2.1 Revision of the Procedure for the Safety Evaluation of Flavouring Agents

The European Food Safety Authority (EFSA) and WHO recently reviewed the general threshold of toxicological concern (TTC) approach in a joint project, building on existing and ongoing work in this area. An expert workshop was convened in December 2014, primarily to provide recommendations as to how the existing TTC framework may be improved and expanded by updating/revising the Cramer, Ford & Hall classification scheme *(6)* and extending the TTC approach. An important aspect was also to develop a globally harmonized decision-tree for a tiered approach on the application of the TTC in the risk assessment of chemicals from oral exposures *(7)*.

Based on the recommendations from this expert workshop, the Committee discussed the consequences for the existing JECFA Procedure for the Safety Evaluation of Flavouring Agents, which is based on the TTC concept, and proposed a revised Procedure (see Fig. 1). The main change proposed is to remove question 2 of the existing Procedure ("Can the substance be predicted to be metabolized to innocuous products?") and in consequence combine the A-side and B-side of the existing Procedure, because:

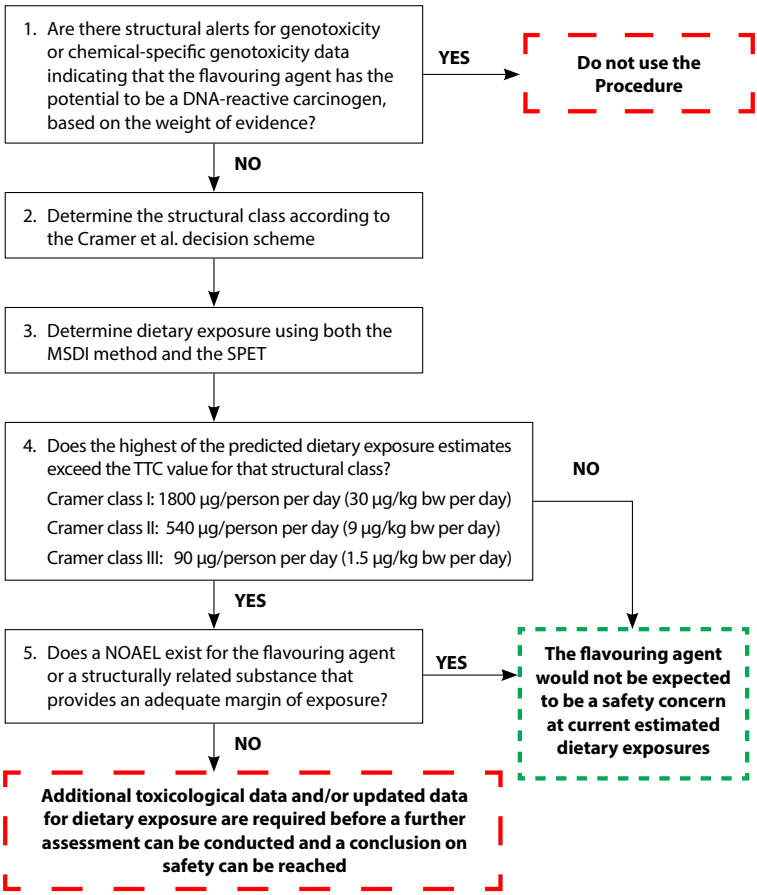
1) metabolism is an inherent part of the Cramer, Ford & Hall classification scheme *(6)* and the TTC values for the different classes;
2) models for predicting metabolism can have significant limitations, including lack of information on interspecies extrapolation and alterations in metabolite profiles arising from saturation of metabolic pathways;
3) prediction of the major pathways of metabolism may not reflect the hazard associated with a minor pathway; and
4) the B-side of the existing Procedure requires toxicity data on the compound or a structurally related substance even if the dietary exposure was below the TTC value, which is inconsistent with the TTC concept.

Another change is to add an initial question regarding genotoxicity and in consequence to delete step B5 ("Do the conditions of use result in an intake greater than 1.5 μg/day?") from the Procedure. The Committee noted that this is the original United States Food and Drug Administration (USFDA) threshold of regulation value of 1.5 μg/person per day, but that this value is of little practical application in the Procedure. Moreover, the Cramer class thresholds as applied would be adequately protective for a non-genotoxic cancer end-point.

The Committee recommends the following points for consideration when deciding on the adequacy of a resulting margin of exposure (MOE) at step 5 of the revised procedure:

- What is the overall strength of the database?
- Is the MOE based on a no-observed-adverse-effect level (NOAEL) for the flavouring agent or for a structurally related substance?
- What is the effect on which the NOAEL is based?
- Is the NOAEL the highest dose tested or identified from a single-dose study?
- What is the duration of the study from which the NOAEL is identified?

Fig. 1
Revised Procedure for the Safety Evaluation of Flavouring Agents



If the overall database is considered, based on expert judgement, to be sufficiently robust, the Committee considered that an MOE that accommodates at least a default uncertainty factor as used in the assessment of food additives may be sufficient to conclude that the flavouring agent would not be expected to be a safety concern at current estimated levels of dietary exposure.

The Committee further concluded that the revised Procedure for the Safety Evaluation of Flavouring Agents should be applied in its future evaluations.

The Committee noted that application of the new Procedure would not have an impact on previous evaluations, because genotoxicity is considered in the current Procedure, metabolism is considered in the Cramer decision-tree and, overall, this new Procedure is equally robust.

2.2.2 Approach for prioritizing flavouring agents for re-evaluation

The Committee at its seventy-ninth meeting (Annex 1, reference *220*) held a preliminary discussion concerning the fact that the submission of additional toxicology data, including genotoxicity data, and/or exposure data for previously evaluated flavouring agents may trigger the need for re-evaluation of previously evaluated flavouring agents. The present Committee reiterated the need for the development of an approach, including a prioritization process, for the re-evaluation of flavouring agents based on all available toxicological data and updated dietary exposure estimates. When developing such an approach, compounds that are used as comparators for structurally related compounds will require specific attention when new data on these become available. The Committee also noted that there is a need to compile data on all flavouring agents that were reported in the monographs of previous meetings and from other sources but not re-evaluated, to assist the prioritization for the re-evaluation.

Moreover, for any flavouring agents for which new toxicological studies are submitted, the sponsor needs to provide updated dietary exposure data.

2.3 Food additive specifications and analytical methods

2.3.1 Replacement of packed column gas chromatographic methods in the specifications monographs

The Committee at its present meeting noted that several specifications monographs contain packed column gas chromatographic methods, which are outdated. The manufacture of packed column gas chromatographs ceased in the early 1990s, and these instruments have been replaced by capillary/wide-bore column gas chromatographs. As a result, analytical methods involving packed column gas chromatographs have become obsolete, which necessitates their replacement.

The Committee recommends that the FAO JECFA Secretariat establish a process to identify the food additive specifications monographs containing packed column gas chromatographic methods and request suitable methods (through a call for data), in order for the Committee to replace these methods in the specifications monographs.

2.3.2 Revision of the FAO JECFA Monographs 1, Combined Compendium of Food Additive Specifications, Vol. 4

The Committee at its present meeting, while developing and revising specifications monographs for food additives, noted that "FAO JECFA Monographs 1, Combined Compendium of Food Additive Specifications, Vol. 4, Analytical methods, test procedures and laboratory solutions used by and referenced in the food additive specifications" was published in the year 2006. Subsequently, several analytical methods associated with the specifications monographs were either

included in the individual monographs or published separately. The Committee also noted that advancement in instrumentation technologies has resulted in the development of several specific, accurate and fast methods since the publication of Volume 4, which necessitates complete revision of Volume 4.

The Committee recommends that the FAO JECFA Secretariat establish a process for the revision of FAO JECFA Monographs 1, Combined Compendium of Food Additive Specifications, Vol. 4.

2.3.3 Limits for lead in specifications of food additives for use in infant formula

The Committee at its seventy-ninth meeting (Annex 1, reference *220*) considered four additives for use in infant formula and formula for special medical purposes intended for infants – namely, carrageenan, pectin, citric and fatty acid esters of glycerol (CITREM) and starch sodium octenyl succinate. At its Eighth Session, the Codex Committee on Contaminants in Foods (CCCF) set a maximum limit of 0.01 mg/kg for lead in infant formula (as consumed) *(8)*. The Committee at the seventy-ninth meeting noted that three of the four food additives considered for risk assessment at that meeting (pectin, CITREM and starch sodium octenyl succinate) could result in exceedance of the maximum limit for lead in infant formula at proposed use levels if lead were present at the specification limits listed in the individual monographs (i.e. at 5 mg/kg in pectin and at 2 mg/kg in both CITREM and starch sodium octenyl succinate). The seventy-ninth JECFA also noted that the introduction of lower lead limits in the specifications (e.g. 1 mg/kg for pectin, 0.5 mg/kg for CITREM and 0.1 mg/kg for starch sodium octenyl succinate) would result in none of these additives exceeding the maximum limit for lead in the final infant formula (i.e. 0.01 mg/kg) if these additives were included in infant formula at the maximum use level reviewed by JECFA.

For the current meeting, data were requested on the levels of lead present in CITREM, pectin and starch sodium octenyl succinate for use in infant formula, and the Committee received data on levels of lead in CITREM and pectin, but not in starch sodium octenyl succinate.

The Committee evaluated the data presented for levels of lead in 12 non-consecutive lots of CITREM. The levels of lead were below 0.1 mg/kg, the limit of quantification of the method (inductively coupled plasma optical emission spectrometry [ICP-OES]), demonstrating that the lead level of 0.5 mg/kg proposed by the seventy-ninth JECFA was achievable for CITREM used in infant formula. The current limit of 2 mg/kg for lead in the CITREM specifications monograph was maintained for general use, and a limit of 0.5 mg/kg was introduced for use in infant formula. The Committee also evaluated data presented for levels of lead in pectin for use in infant formula analysed by two different analytical methods. Levels reported for lead in 12 non-consecutive lots

of pectin analysed by inductively coupled plasma atomic emission spectrometry (ICP-AES) were below the limit of detection of the method (0.4 mg/kg). The mean level of lead reported for five non-consecutive lots of pectin analysed by inductively coupled plasma mass spectrometry (ICP-MS) was 0.017 mg/kg. Based on the data provided, the Committee noted that the levels of lead in pectin intended for use in infant formula were below the level of 1 mg/kg considered by the Committee at the seventy-ninth meeting. The current limit of 5 mg/kg for pectin in the specifications monograph was reduced to 2 mg/kg for general use, and a limit of 0.5 mg/kg was introduced for use in infant formula.

The Committee also considered the levels of lead in the specifications monographs of two other additives on the agenda for consideration for use in infant formula – namely, carob bean gum and xanthan gum – in light of this discussion. Based on the data provided, the Committee maintained the lead limits in the specifications monographs for these two additives for general use (2 mg/kg) and introduced lead limits of 0.5 mg/kg for use in infant formula.

Based on the data submitted for CITREM, pectin, carob bean gum and xanthan gum, the Committee was reassured that the overall criterion for lead levels in the ingredients for use in infant formula is achievable. However, the Committee further reaffirmed that it is the responsibility of the infant formula manufacturers to ensure that the lead levels in the final infant formula (as consumed) comply with the maximum limit for lead as set by the Eighth Session of CCCF.

The Committee recommended that all additives (including starch sodium octenyl succinate) for use in infant formula be reviewed for lead levels in the specifications.

2.3.4 Use of chloroform as solvent in the test methods associated with specifications monographs for synthetic colours

The Committee at its present meeting, while revising specifications monographs for synthetic food colours, noted that chloroform, a solvent restricted in several modern laboratories due to safety concerns, is used as a solvent in the test methods associated with the determination of total dye content in organic solvent–soluble colouring matter and subsidiary colouring matter. The Committee previously made a similar recommendation to phase out the use of chloroform.

The Committee recommends the development of analytical methods with suitable replacement solvent(s), in order to replace chloroform, in the future.

2.4 Flavour specifications

2.4.1 General inclusion of infrared spectra

While preparing specifications for the new flavouring agents, the Committee noted that less than half of the specifications prepared at the current meeting included an infrared spectrum as one of the available reference spectra for the purpose of an identification test. Infrared spectroscopy is a simple, yet useful, method that is widely used by the flavouring industry to serve as an identification test for flavouring agents. The Committee recommended that all future specifications for new flavouring agents contain a high-quality readable infrared spectrum in the data submission.

2.4.2 Inclusion of chemical structures in the JECFA flavourings database

While preparing specifications for the new flavouring agents, the Committee noted that chemical structures for the flavouring agents are not included as part of the specifications. The chemical structures are reviewed during the evaluation of the flavourings, but are included only as part of the toxicological evaluation of the flavouring and are not captured in the specifications. The availability of the chemical structure for the flavourings in the flavourings specifications database would be of great value to users of the database. The Committee recommended that chemical structures be included in the JECFA flavourings database.

3. Specific food additives (other than flavouring agents)

The Committee evaluated one food additive for the first time and re-evaluated six others. In addition, the Committee evaluated the safety of three previously evaluated food additives for use in infant formula and formula for special medical purposes intended for infants. Twenty-two food additives (including 16 modified starches) 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[2]

3.1.1 Allura Red AC

Explanation

Allura Red AC (INS No. 129) is a monoazo dye that is widely used as a synthetic food colour in many countries around the world.

The Committee previously evaluated Allura Red AC at its eighteenth, twenty-third, twenty-fourth and twenty-fifth meetings (Annex 1, references *35*, *50*, *53* and *56*). At its twenty-fourth meeting, the Committee established a temporary ADI of 0–7 mg/kg body weight (bw) based on long-term rat studies. At its twenty-fifth meeting, the Committee established a full ADI of 0–7 mg/kg bw.

At the present meeting, the Committee re-evaluated Allura Red AC at the request of the Forty-seventh Session of CCFA [1]. In response to the Committee's request for further data on Allura Red AC, new studies on biochemical effects, genotoxicity, reproductive and developmental toxicity, neurobehavioural effects and observations in humans were submitted. The Committee also considered other related information retrieved from a literature search.

The previous monograph has been expanded and is reproduced in a consolidated monograph. References from 1980 onward were not considered by previous Committees.

Chemical and technical considerations

Allura Red AC (INS No. 129) is allowed as a food colour in the European Union (EU), Japan, the USA and other regions. It is used for colouring beverages, frozen treats, powder mixes, gelatine products, candies, icings, jellies, spices, dressings, sauces, baked goods and dairy products.

[2] Numbered references cited in the subsections of section 3.1 are provided at the end of each subsection.

Allura Red AC consists mainly of disodium 6-hydroxy-5-(2-methoxy-5-methyl-4-sulfonato-phenylazo)-2-naphthalene-sulfonate and subsidiary colouring matters together with sodium chloride and/or sodium sulfate as the principal uncoloured components. It is manufactured by coupling diazotized 4-amino-5-methoxy-2-methylbenzenesulfonic acid with 6-hydroxy-2-naphthalene sulfonic acid. The resulting dye is purified and isolated as the sodium salt. Specified impurities include uncombined starting materials, subsidiary colouring matters related to the primary dye component, unsulfonated primary aromatic amines and lead.

Biochemical aspects

Allura Red AC is poorly absorbed in rats and dogs, with up to 95% of the total intake being excreted in the faeces [2, 3]. Cresidine sulfonic acid was found to be the major metabolite of Allura Red AC in both the urine and faeces of rats and dogs [4]. No new metabolic or kinetic studies have become available since the last evaluation by the Committee.

Toxicological studies

Allura Red AC has low oral acute toxicity in mice [5], rats [6], rabbits [7] and dogs [8]. Short-term studies of toxicity of Allura Red AC in several species, including rats [9], dogs [10, 11] and pigs [12], revealed no compound-related effects other than coloration of the urine and faeces. No new short-term studies have become available since the last evaluation by the Committee.

In several long-term studies of toxicity and carcinogenicity, mice or rats were fed Allura Red AC in the diet at a level of 0%, 0.37%, 1.39% or 5.19%. The first of two studies in mice was suggestive of an earlier onset of lymphatic tumours, but this was not confirmed in the second, more extensive study [13–15]. In two rat studies, the only effect seen was decreased body weight at the highest dose tested. No evidence of carcinogenicity was observed in these studies. Based on the reduced body weight in both sexes in one study [16] and in females in another study [17] observed at 5.19% Allura Red AC in the diet, the NOAEL was 1.39% (equivalent to 695 mg/kg bw per day, calculated using default dose conversion factors). These long-term studies were available for evaluation by the previous Committee as unpublished study reports. The present Committee noted that one study [17] was later published in the scientific literature and that the NOAEL of 1.39% in feed was calculated to be equal to 901 mg/kg bw per day, based on measured feed consumption and body weight data [18].

No evidence for genotoxic potential of Allura Red AC was found in numerous in vitro mutagenicity studies [19–27] or in vivo assays [28–33]. Both Allura Red AC and the expected metabolic products, sulfonated naphthylamines

that are formed in vivo by azo-reduction, did not reveal any genotoxic potential in vitro [34].

The only indication of potential genotoxicity of Allura Red AC was DNA damage in cells of the colon and the glandular stomach of mice, but not of rats, reported by one group of researchers, using the comet assay [5, 35, 36]. Such DNA damage in mice could not be confirmed by other studies conducted according to Organisation for Economic Co-operation and Development (OECD) guidelines [32, 33]. Therefore, the overall evidence demonstrates that Allura Red AC is not genotoxic.

In mice, no reproductive toxicity at dose levels up to 2520 mg/kg bw per day over two generations was reported [37]. In a two-generation reproductive toxicity study in rats [38], slight growth suppression was observed in F_1 and F_2 pups at 5.19% Allura Red AC in the diet, the highest concentration tested, as well as in the low-dose group of the F_{1B} generation. In the absence of detailed original data, the toxicological relevance of the observed "slight growth suppression" could not be assessed.

In a study in rats [39], reduced reproductive success and reduced cerebellar weight in the offspring of all treated animals were observed. The reported effects in this study showed no dose–response relationship. In addition, the two long-term mouse studies [13–15] and one lifetime rat study described above [17, 18] included an in utero exposure phase. No treatment-related reproductive or developmental toxicity was observed in the two studies in mice; the NOAEL was 5.19% in the diet (equal to 7318 mg/kg bw per day) [15]. For the study in rats, the NOAEL for general toxicity was 1.39% (equal to 901 mg/kg bw per day), based on the body weight reduction observed in females at 5.19% [18].

Developmental toxicity studies in rats [40–42] and rabbits [43] did not show any compound-related embryotoxic or teratogenic effects. A statistically significant increase in the incidence of reduced ossification of the hyoid was noted at the high dose level of 0.7% in the drinking-water (equal to 939 mg/kg bw per day) in a study in rats [44], but no significant effect on hyoid bone was seen in a parallel study by the same authors at doses up to 1000 mg/kg bw per day [42]. The reduced ossification of the hyoid observed in one study was therefore considered to be an incidental finding of no toxicological relevance, and the NOAEL was 1000 mg/kg bw per day, the highest dose tested.

A special study found that Allura Red AC inhibited aromatase activity in vitro [45]. The Committee noted that although aromatase has been implicated as a target for endocrine disrupting chemicals, considering the limited systemic bioavailability of Allura Red AC from the oral route and the absence of reproductive and developmental toxicity in other studies, this finding has no toxicological relevance.

Neurobehavioural effects were reported in some special studies. In the two-generation study in mice described above, no neurobehavioural effects were found at dose levels up to 2520 mg/kg bw per day [37]. In the one-generation study in rats reported above [39], decreased running wheel activity was reported at all dose levels, but did not show a dose–response relationship. Neurobehavioural effects were reported in rats treated with mixtures of colours including Allura Red AC [46–49]. However, the use of mixtures in these studies does not permit any observed effects to be ascribed to individual components, including Allura Red AC.

Observations in humans

The Committee noted that it had previously considered a study that investigated the possibility of a relationship between hyperactivity in children and the consumption of beverages containing a mixture of food colours, including Allura Red AC, and a preservative, sodium benzoate [50]. As concluded previously by the Committee (Annex 1, reference *206*), this study was of limited value because of inconsistencies in the findings and the use of mixtures of food colours.

There were reports suggesting the observation of urticaria/angio-oedema [51] and vasculitis [52] after dietary exposure of human subjects to Allura Red AC. However, the first [51] study was characterized by poorly controlled challenge procedures, and only one patient was reported in the second study, which involved consumption of a mixture with other synthetic colours [52]. Additionally, sensitivity to food colours in patients with chronic urticaria/angio-oedema or asthma was uncommon in better controlled studies [53, 54].

Assessment of dietary exposure

Estimates of dietary exposure to Allura Red AC prepared and published by EFSA, the USFDA and Food Standards Australia New Zealand (FSANZ) were available to the Committee, in addition to published papers for the Korean and Kuwaiti populations and information from industry. The study of schoolchildren in Kuwait was not further considered by the Committee, as it was not nationally representative.

The Committee concluded that EFSA's 95th percentile exposure estimate for European children aged 3–9 years of 0.9–2.9 mg/kg bw per day for brand-loyal consumers represented the most conservative estimate based on extensive reported and/or industry use data across all countries and age groups assessed [55]. Available data on estimates of dietary exposure to Allura Red AC for children who were high consumers based on analytical data from other countries were of a similar magnitude, but slightly lower than the EFSA estimate: for the Australian population aged 2–16 years, 0.03–0.04 mg/kg bw per day (90th

percentile consumers) [56]; and for the USA population aged 2–5 years and 13–15 years, 0.13–0.27 mg/kg bw per day (90th percentile consumers) [57]. For the Korean population aged 1 year and over, estimated dietary exposure was 0.7 mg/kg bw per day (95th percentile consumers) [58, 59].

The Committee concluded that estimates of dietary exposure to Allura Red AC for different countries utilized the same approach and were comparable and that estimated dietary exposures ranging from 0.03 to 2.9 mg/kg bw per day for children who were high consumers should be used for the safety assessment of Allura Red AC.

Evaluation

The existing ADI of 0–7 mg/kg bw is based on a NOAEL of 1.39% in the diet derived from three rat studies [16, 17, 38]. The NOAEL was equivalent to 695 mg/kg bw per day, using default dose conversion factors. Although the NOAEL for one of these studies has been recalculated to a higher value of 901 mg/kg bw per day, using measured feed consumption and body weight data [18], it is not possible to recalculate the NOAEL for the other rat study [16]. Therefore, the Committee concluded that the new data do not give reason to revise the ADI and confirmed the ADI of 0–7 mg/kg bw. The Committee noted that the range of estimated dietary exposures to Allura Red AC for children based on reported and/or industry use data, including the conservative estimate by EFSA, were below the upper bound of the ADI (0.4–41%). The Committee concluded that dietary exposure to Allura Red AC for children and all other age groups does not present a health concern.

A consolidated monograph was prepared.

Specifications were prepared at the twenty-eighth meeting of JECFA (Annex 1, reference *66*), and metals and arsenic specifications were revised at the fifty-ninth meeting (Annex 1, reference *160*). At the present meeting, the method for the determination of lead was changed from atomic absorption to any method appropriate to the specified level. Updated high-performance liquid chromatography (HPLC) conditions were added for determining subsidiary colouring matters and organic compounds other than colouring matters. The method of assay was changed to visible spectrophotometry, and spectrophotometric data were provided for the colour dissolved in water.

The existing specifications were revised, and a Chemical and Technical Assessment was prepared.

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3.1.2 Carob bean gum

Explanation

Carob bean gum (INS No. 410) is used as a thickener, stabilizer, emulsifier and gelling agent. Carob bean gum as a food additive was evaluated by the Committee at its thirteenth, eighteenth, nineteenth, twenty-fourth and twenty-fifth meetings (Annex 1, references *19*, *35*, *38*, *53* and *56*). A temporary ADI "not specified" was established at the nineteenth meeting in 1975, and the temporary status was extended at the twenty-fourth meeting in 1980. When additional toxicity studies became available, a full ADI "not specified" was established at the twenty-fifth meeting of the Committee in 1981. Current specifications were established by the Committee at its sixty-seventh meeting (Annex 1, reference *184*).

At the request of CCFA at its Forty-seventh Session [1], the Committee evaluated the safety of carob bean gum for use as thickener in infant formula and formula for special medical purposes intended for infants in the context of the (therapeutic) dietary management of gastro-oesophageal reflux. The Committee notes that ADIs do 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. The proposed use level of carob bean gum is up to 10 000 mg/L for infant formula. The sponsor suggested that a typical use level would be 5000 mg/L.

Data submitted for the evaluation included information related to microbial fermentation in the gastrointestinal tract, acute and short-term toxicity studies in animals, in vitro genotoxicity studies, special studies in newly weaned

pigs, and published infant growth and tolerability trials. A literature search was also conducted.

Chemical and technical considerations

Carob bean gum (also known as locust bean gum [LBG], carubin and algarroba; INS No. 410; Chemical Abstracts Service [CAS] No. 9000-40-2; European Inventory of Existing Commercial Chemical Substances [EINECS] 232-541-5) and carob bean gum (clarified) consist mainly of high molecular weight (in the range of 50–3000 kDa) galactomannans. Carob bean gum consists of a linear chain of (1→4)-linked β-D-mannopyranosyl units (mannopyranose) with (1→6)-linked α-D-galactopyranosyl residues (galactopyranose) as side-chains. The mannose to galactose ratio of carob bean gum is approximately 4:1. The mannose and galactose contents have been reported as 73–86% and 27–14%, respectively. Galactomannans are also commonly found in other gums, such as guar, tara or cassia gum, but with different mannose to galactose ratios.

Carob bean gum has the capacity to form very viscous solutions at relatively low concentrations, which are almost unaffected by pH, salts or temperature. It is commonly used as a food additive for its thickening, stabilizing, emulsifying or gelling properties. Its thickening properties have been employed in infant formulas for the dietary management of infant regurgitation for more than 20 years in countries of the EU.

Carob bean gum is obtained from the endosperm of the seed of the carob (locust) tree, *Ceratonia siliqua* (L.) Taub (Fam. Leguminosae). The seeds are dehusked by treatment with dilute sulfuric acid or by thermal mechanical treatment, elimination of the germ followed by milling and screening of the endosperm (native carob bean gum). The gum may be washed with ethanol or isopropanol to control the microbiological load (washed carob bean gum). Native carob bean gum may also be further clarified by dispersing in hot water, recovery with isopropanol or ethanol, filtering, drying and milling, which is known as clarified carob bean gum.

The sponsor, in the dossier submitted for the present meeting, identified a cold-soluble carob bean gum for use in infant formula. The Committee was not able to consider this product from a chemical and technical point of view because limited information about its manufacture and no data about its composition were received.

Biochemical aspects

The Committee previously concluded that carob bean gum is a non-digestible galactomannan that is not bioavailable or hydrolysable, but noted that some decrease in chain length may occur through fermentation by microflora in the

gut. These properties have been reported for other related galactomannans with varying mannose to galactose ratios previously evaluated by the Committee: guar gum, cassia gum and tara gum (Annex 1, references *39*, *62* and *74*, respectively).

Increased microbial activity in association with increased caecum weights and caecum content weights were observed in rats fed a diet containing carob bean gum at 50 g/kg (equivalent to 2500 mg/kg bw per day) for 28 days. This observation supports the conclusion that fermentation of carob bean gum occurs in the gastrointestinal tract of rats [2]. Fermentation of carob bean gum by microbiota in the gut produces oligosaccharides or monosaccharides, which will be further converted to short-chain fatty acids; these short-chain fatty acids can be absorbed and metabolized in normal biochemical pathways.

Toxicological studies

In previous evaluations, the Committee found no adverse effects in short-term toxicity or long-term toxicity and carcinogenicity studies in rats or mice or in reproductive toxicity studies in rats. Carob bean gum gave negative results in several mutagenicity assays. Dogs fed diets containing 10% carob bean gum for 30 weeks exhibited hypermotility, soft, bulky stools and reduced digestibility.

At the current meeting, two short-term studies, not previously evaluated, in which rats were fed carob bean gum at either 5% or 8% (equivalent to 2500 or 4000 mg/kg bw per day) in the diet for 28 days or 14 days, respectively, were reviewed [2, 3]. Caecal enlargement was noted in both studies, but the Committee concluded that the effect is not toxicologically relevant, as it is considered to be an adaptive response in rodents administered diets containing high levels of indigestible carbohydrates. A statistically significant reduction in body weight (<10%) of rats fed 8% carob bean gum for 14 days was observed, but feed consumption was not measured.

In another short-term study not previously evaluated, no adverse effects were observed in mice or rats fed carob bean gum at concentrations up to 100 000 mg/kg feed (equivalent to 15 000 and 10 000 mg/kg bw per day, respectively) for up to 90 days. No effects on body weight were observed in either rats or mice [4].

Carob bean gum gave negative results in a bacterial reverse mutation assay, with and without metabolic activation, in *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535 and TA1537 [5]. The Committee concluded that carob bean gum is not mutagenic.

Special studies

The current Committee evaluated several studies that measured the potential of carob bean gum to decrease the bioavailability of minerals using an in vitro continuous flow dialysis system that simulated the upper gastrointestinal tract of

infants less than 6 months of age. These studies demonstrate that infant formulas with a carob bean gum concentration higher than 4000 mg/L may reduce the levels of calcium, zinc and iron available for absorption [6–9].

Newly weaned 5-week-old piglets were fed a control diet, a 1% carob bean gum diet (equal to 240 mg/kg bw per day) or a 10% carob tree meal diet containing approximately 50% carob bean gum (equal to 1272 mg/kg bw per day) for 11 or 12 days to investigate the bacteriological and morphological characteristics of the small intestine of piglets fed the test substance. The Committee calculated the daily dose levels using the feed consumption and body weight data reported by the authors. Weight gain and daily feed intake were similar for all of the groups. No significant effects on intestinal morphology or histological parameters were observed in piglets fed 1% carob bean gum, with the exception of slight changes in the mitotic index of the crypts when compared with controls. The absence of histopathological changes suggests that cell renewal balance of intestinal mucosa (death/proliferation) is not occurring at this dose. Addition of 10% carob tree meal to the diet affected the bacteriological and morphological characteristics of the small intestine. The 10% carob tree meal diet contained a significant portion of unidentified components, including polyphenols with antibacterial properties, which makes attributing these effects to carob bean gum difficult. The NOAEL for this study was 1% carob bean gum (equal to 240 mg/kg bw per day) [10]. The Committee noted that the newly weaned piglet model is not a neonatal animal model and may not mimic the infant gut at 0–12 weeks.

The effects of carob bean gum on immunological parameters of intestinal function were also measured in a study using 4-week-old newly weaned piglets. Piglets were fed the experimental diet containing 0.5% carob bean gum ad libitum for 14 days, followed by *E. coli* oral challenge infection to measure the immune response by monitoring C-reactive protein, immunoglobulin A (IgA) levels in blood and Toll-like receptors TLR2 and TLR4 mRNA levels in the ileum and mesenteric lymph node. No statistically significant differences were observed in IgA or TLR expression between treatment groups. A statistically significant repression of C-reactive protein induction after *E. coli* challenge was observed in piglets fed the carob bean gum–containing diet, indicative of a reduction in the acute inflammatory response caused by the challenge [11].

Observations in humans

No untoward gastrointestinal effects in adults or infants were observed in feeding studies previously evaluated by the Committee.

Thirteen new paediatric trials in healthy term infants were evaluated by the current Committee. In these, formula thickened with carob bean gum was compared with either standard infant formula or formula thickened with another substance. Trials generally focused on growth, formula intake, regurgitation

events, and volume and stool characteristics. In all, about 400 term infants were assessed in trials ranging from 1 week to 3 months at concentrations of carob bean gum ranging from 3500 to 6000 mg/L. Formulas were generally well tolerated, and no effects on growth were reported in any of the trials. Reduced frequency of regurgitation was often observed. In one study, there was no difference in gastric emptying time in infants fed commercial formula thickened with carob bean gum [12]. None of the studies reported statistically significant levels of severe gastrointestinal effects such as diarrhoea, but some did report increased bowel movements in infants receiving formula thickened with carob bean gum. Overall, the Committee concluded that the results from these studies did not reveal any serious adverse effects and generally showed the formula to be well tolerated.

One of the above paediatric trials, a randomized, prospective study in healthy infants, addressed the potential concerns for reduced mineral and nutrient bioavailability of carob bean gum–thickened formula suggested by the in vitro studies. Infant formula was fed to 20 healthy infants who received either a control whey-predominant formula or a casein-based formula containing carob bean gum at 4000 mg/L for 13 weeks [13]. All infants grew normally; infant weight was slightly higher in infants fed the carob bean gum–containing formula, but the difference was not significant. Iron, calcium, phosphorus, iron binding capacity and zinc levels in blood were measured, along with total serum albumin, pre-albumin and urea at the end of the study. All serum parameters, including those related to minerals, were comparable between the control and test groups when evaluated at the end of the study. Slight, statistically significant differences between the groups were observed in the levels of urea and albumin, which the authors attributed to the differences between the casein- and whey-based formulas.

A single case of allergenicity was reported for one 5-month-old infant following exposure to carob bean gum. The infant, with previously identified hypersensitivity reactions, exhibited explosive vomiting, urticaria and a facial rash following exposure to carob bean gum–thickened formula. A fluorescent allergosorbent test confirmed a positive reaction to carob bean gum [14]. A single isolated adult case report of carob bean gum hypersensitivity has also been published [15]. Fiocchi et al. [16] investigated the potential for carob bean gum to induce an immune response in 12 peanut-allergic children. Although some participants produced an IgE-specific response and some were positive for a skin prick test using carob meal, there was no clinical reactivity with either raw or cooked carob during the double-blind placebo-controlled food challenges for any of the patients.

The Committee noted two case reports of isolated adverse events in extremely low birth weight infants fed formula containing carob bean gum [17, 18], but concluded that the effects could not be attributed to carob bean gum.

Assessment of dietary exposure

The maximum proposed use level for carob bean gum in infant formula is 10 000 mg/L.

Infant formula consumption estimates were derived from mean 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 per kilogram body weight 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 needs to the volume of formula ingested daily.

Dietary exposure to carob bean gum from its use at the proposed use level in infant formula ranges from 600 to 1800 mg/kg bw per day in infants aged 0–12 weeks, whereas infants with high (95th percentile) energy intakes may reach an exposure level of 2200 mg/kg bw per day.

Evaluation

The Committee previously assigned an ADI "not specified" to carob bean gum, but this 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. Therefore, special considerations are required for this age group on a case-by-case basis, and toxicological testing strategies for additives to be used in infant formulas require different approaches, including studies involving exposure of very young animals. The Committee previously concluded that studies incorporating direct oral administration to neonatal animals are required for the evaluation of food additives in infant formula (Annex 1, reference *220*).

Data available for the evaluation of carob bean gum include studies in adult animals, reproductive and developmental toxicity studies that did not include direct oral administration during the neonatal phase, and a special study in newly weaned piglets that are 5 weeks of age, which is beyond the neonatal period. Human infant feeding studies evaluated by the Committee do not report any serious adverse effects and support tolerability up to 6000 mg/L, but are not designed to evaluate effects on infant gut morphology or health.

The Committee concluded that these studies are not sufficient for the evaluation of carob bean gum for use in infant formula at the proposed use level. The Committee requests toxicological data from studies in neonatal animals, adequate to evaluate the safety for use in infant formula, to complete the evaluation.

An addendum to the monograph was prepared.

The Committee discussed the issue of contribution of lead from the use of carob bean gum at the proposed levels in infant formula (see section 2.3.3). The Committee introduced a limit for lead of 0.5 mg/kg for use in infant formula in the specifications monograph. There were insufficient data to set a limit for arsenic.

The Committee also updated the method for the determination of lead and the sample preparation for residual solvents in the specifications monographs.

The Committee noted that the current use level of carob bean gum for infant formula or for formula for special medical purposes intended for infants in CODEX STAN 72-1981 [19] (1000 mg/L) is much lower than the proposed use level (10 000 mg/L).

The Committee noted that the sponsor also identified a cold-soluble carob bean gum for use in infant formula. However, no information was provided on the manufacturing and composition of the product, and the Committee was unclear which product is used in infant formula and formula for special medical purposes intended for infants.

The existing specifications of carob bean gum and carob bean gum (clarified) and the Chemical and Technical Assessment were revised.

The Committee recommended that all additives for use in infant formula be reviewed for arsenic levels in the specifications.

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3.1.3 Lutein esters from *Tagetes erecta*

Explanation

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 seventy-ninth meeting (Annex 1, reference *220*), the Committee evaluated lutein esters from *Tagetes erecta*. The Committee noted that limited information was received on the manufacturing process for and composition of lutein esters from *Tagetes erecta* and therefore prepared tentative specifications for the product. Based on the available toxicological data, including newly submitted studies, and a dietary exposure assessment, the Committee concluded that there was no need to establish a numerical ADI. 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.

Chemical and technical considerations

Lutein esters from *Tagetes erecta* L. are an organic solvent extract derived from the dried petals of yellow marigold flowers. The preparation contains lutein esters of which lutein dipalmitate accounts for the major part; a smaller proportion of zeaxanthin esters is also present. Lutein dipalmitate (Helenien; β,ε-carotene-3,3′-diol dipalmitate) is a member of the xanthophylls group of pigments and has no provitamin A activity. The balance of the extract is made up of naturally occurring waxes.

Lutein esters are used as a food colour and nutrient supplement in a wide range of baked goods and baking mixes, beverages and beverage bases, breakfast cereals, chewing gum, dairy product analogues, egg products, fats and oils, frozen dairy desserts and mixes, gravies and sauces, soft and hard candy, infant and toddler foods, milk products, processed fruits and fruit juices, and soups and soup mixes at levels ranging from 2 to 330 mg/kg.

Evaluation

At the present meeting, the Committee received analytical data for five batches of lutein esters from *Tagetes erecta* with details on the composition of the carotenoid portion. Based on the analytical data submitted, the assay value was increased from 60% to 75% for total carotenoids. The Committee included in the specifications a method for the determination of the proportion of zeaxanthin

in total carotenoids (<10%). The Committee also received sufficient information about the non-carotenoid portion of the extract to set an upper limit of 25% for waxes present in the product in commerce. The Committee also made necessary amendments to the method for the determination of the waxes.

The tentative specifications were revised, and the tentative status was removed. A revised Chemical and Technical Assessment was prepared.

The Committee removed the temporary designation from the ADI "not specified" because the tentative status of the specifications was removed and established an ADI "not specified" for lutein esters from *Tagetes erecta*.

The Committee at its seventy-ninth meeting 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. The current Committee was not able to consider this aspect in detail and recommends that this be taken up at a future meeting.

An addendum to the monograph was not prepared.

3.1.4 Octenyl succinic acid (OSA)–modified gum arabic

Explanation

Octenyl succinic acid (OSA)–modified gum arabic (INS No. 423) is intended to replace gum arabic as an emulsifier in a number of food applications. At its seventy-first meeting (Annex 1, reference *196*), the Committee established a temporary ADI "not specified" for OSA-modified gum arabic, on the basis of the available data indicating very low toxicity, comparable with the toxicity of traditional gum arabic and starch sodium octenyl succinate (OSA-modified food starch). As there were no experimental data available at that time on the de-esterification of OSA-modified gum arabic, the Committee made the ADI temporary, pending submission of data 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. New specifications for OSA-modified gum arabic were prepared at that meeting.

At its seventy-fourth meeting (Annex 1, reference *205*), the Committee evaluated new data on the hydrolysis of OSA-modified gum arabic and concluded that the results from the experiments 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 requested that additional data be provided (data on the stability of OSA-modified gum arabic in food and data showing complete hydrolysis in the gastrointestinal tract) by the end of 2013 for further evaluation. 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 [1]. Considering that spontaneous hydrolysis of OSA-modified gum arabic in water was unlikely to occur, the Committee doubted the validity of the observed hydrolysis in the presence of gastrointestinal enzymes. As the study did not unequivocally demonstrate that OSA-modified gum arabic hydrolyses completely in the stomach into gum arabic and OSA, the validity of using toxicological data on gum arabic in the evaluation of OSA-modified gum arabic was not confirmed. The Committee also reviewed data on the stability of OSA-modified gum arabic in food. Although these data demonstrated that OSA-modified gum arabic provided a stable emulsion in the two model food systems evaluated, the data did not unequivocally demonstrate that the OSA-modified gum arabic, at the molecular level, is stable in food and beverages. 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. The specifications were made tentative, pending submission of information on an analytical method to measure the degree of substitution and the results of the analysis of at least five commercially available batches.

At the seventy-ninth meeting (Annex 1, reference *220*), after evaluating a demulsification study in simulated gastric fluid, simulated intestinal fluid and water using emulsions prepared with OSA-modified gum arabic, the Committee was of the opinion that the study did 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 was not confirmed. The Committee also evaluated data on the chemical composition of OSA-modified gum arabic in commerce and noted that the residual (free) OSA was in the range of 3–4% (weight per weight), which is not in accordance with the existing specifications of a value not higher than 0.3%. Furthermore, the submitted data did not clarify the nature of the linkage between the OSA and the gum. The Committee therefore maintained the tentative status of the specifications and decided that the temporary ADI "not specified" would be withdrawn unless adequate data to complete the safety evaluation were submitted by the end of 2015.

At the present meeting, the Committee evaluated an additional study on the hydrolysis of OSA-modified gum arabic in simulated gastric fluid and

simulated intestinal fluid [2], as well as an in silico structure–activity relationship analysis of OSA-modified galactose [3]. The Committee also evaluated data on the manufacturing process, including the use of processing aids, chemical characterization of the product in commerce and updated analytical methods for the determination of esterified (bound) and residual (free) OSA.

Chemical and technical considerations

OSA-modified gum arabic is intended to replace gum arabic as an emulsifier in a number of food applications, but at lower concentrations (approximately half). The introduction of lipophilic groups to the polysaccharide in gum arabic results in enhanced emulsifying properties for OSA-modified gum arabic relative to the parent compound.

OSA-modified gum arabic, produced by esterifying gum arabic (*Acacia seyal* or *Acacia senegal*) in aqueous solution with not more than 3% of octenyl succinic acid anhydride, has been previously reviewed by the Committee at its seventy-first, seventy-fourth, seventy-seventh and seventy-ninth meetings (Annex 1, references *196*, *205*, *214* and *220*).

Evaluation of the newly submitted studies

In the new hydrolysis study, OSA-modified gum arabic was incubated with water, simulated gastric fluid or simulated intestinal fluid at 37 °C for 1.5 hours and with simulated gastric fluid for 4 hours. The free OSA released during incubation of OSA-modified gum arabic was determined by an HPLC method developed by Qiu et al. [4], whereas total OSA content was determined by hydrolysis of the ester linkage of OSA-modified gum arabic using sodium hydroxide, followed by HPLC determination of total OSA. After incubation of OSA-modified gum arabic (3 mg/mL) with water, simulated gastric fluid or simulated intestinal fluid for 1.5 hours, the per cent free OSA was found to be 21%, 43.5% and 2.9%, respectively. When incubation with simulated gastric fluid was extended to 4 hours, the per cent free OSA was found to be 84%. A lower concentration of OSA-modified gum arabic (0.3 mg/mL) incubated with simulated gastric fluid for 4 hours resulted in 87.7% free OSA. The Committee noted that normal gastric emptying in humans is reported to be between 2 and 5 hours [5]. The new hydrolysis data show that OSA-modified gum arabic is hydrolysed up to 88% to gum arabic and OSA within 4 hours.

The new data showed 21% hydrolysis after 1.5 hours in water, which is not in accordance with the claimed stability of the OSA-modified gum arabic in the two model food systems (beverage and salad dressing emulsions).

A structure–activity relationship analysis was performed on OSA-modified galactose by using DEREK for Windows software (Lhasa Ltd, version

11.0 updated 2008) [3], which identified no structural alerts that would predict toxicity. The Committee considered this analysis as not relevant to address the questions raised by the previous Committee.

Assessment of dietary exposure

At its seventy-first meeting, the Committee evaluated dietary exposure to OSA-modified gum arabic, and no new information was available at the present meeting. The current Committee reviewed the previous evaluation to ensure that it remains current. The Committee at its seventy-first meeting used national estimates of dietary exposure to OSA-modified gum arabic that ranged up to 17 mg/kg bw per day for high-percentile consumers to conservatively set dietary exposure for risk assessment purposes at "less than 20 mg/kg bw per day". The current Committee concluded that this estimate remains valid.

Evaluation

The previous Committee questioned the validity of the hydrolysis study available at the time. The present Committee noted that the new hydrolysis data in simulated gastric fluid showed that OSA-modified gum arabic is hydrolysed up to 88% to gum arabic and OSA within 4 hours. The Committee concluded that the studies of short-term toxicity with OSA-modified gum arabic and the read-across from toxicity data on gum arabic, evaluated at previous meetings, do not raise toxicological concerns. Therefore, the Committee removed the temporary designation and established an ADI "not specified" for OSA-modified gum arabic.

No addendum to the monograph was prepared.

The existing tentative specifications were revised, and the tentative status was removed. The Chemical and Technical Assessment was not revised.

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3.1.5 Pectin

Explanation

Pectins (INS No. 440; CAS No. 9000-69-5) are used as gelling, thickening and stabilizing agents. Pectins as food additives have been evaluated by the Committee at its thirteenth, seventeenth, eighteenth, nineteenth, twenty-fifth and seventy-ninth meetings (Annex 1, references *19*, *32*, *35*, *38*, *56* and *220*). At its twenty-fifth meeting in 1981, the Committee established a group ADI "not specified" for pectin and amidated pectin.

At its seventy-ninth meeting, the Committee evaluated data relevant to the safety of pectin in infant formula, noting that the group ADI 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. The Committee concluded that estimated exposure to pectin from its use in infant formula (1100 mg/kg bw per day) at the then proposed level of 0.5% (5000 mg/L) was in the region of the NOAEL of pectin (847 mg/kg bw per day) and close to the lowest-observed-adverse-effect level (LOAEL) (3013 mg/kg bw per day), based on decreased feed intake and body weight gain in a neonatal pig study. 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 therefore concluded that the use of pectin in infant formulas at the maximum proposed use level (0.5%) was of concern and 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. In addition, the Committee requested data on levels of lead when the additive is intended for use in infant formula.

At the present meeting, the Committee was asked to consider the additional data provided in support of the safety of pectin in infant formula and formula for special medical purposes intended for infants at the reduced maximum proposed use level of 0.2% (2000 mg/L). In response to the Committee's request for data, a dossier containing a revised report and reanalysis of the neonatal pig study evaluated by the Committee at the seventy-ninth meeting and an additional study on pectin in neonatal pigs was submitted for evaluation.

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 pectin used in food will vary depending upon the pectin source and processing and is expected to range from 100 to 200 kDa.

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.

Biochemical aspects

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.

Pectin-derived acidic oligosaccharides (pAOS) are a product of the digestion of food-grade pectin and consist 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 at the seventy-ninth meeting concluded that studies on pAOS can support conclusions reached on the basis of data from studies that have used pectin.

Toxicological studies

At the seventy-ninth meeting, data on pectin and pAOS relevant to the safety assessment of the use of pectin in infant formula and formula for special medical purposes intended for infants were evaluated. The Committee concluded that the NOAEL of pAOS from short-term toxicity studies in rats was about 7000 mg/kg bw per day, the highest dose tested, and concluded that pAOS is not genotoxic. Decreased feed intake and body weight gain were reported at 1.0% (reported to be equal to 3013 mg/kg bw per day) in neonatal pigs fed pectin-containing milk replacer. Although no overt toxicological effects were observed in this study, decreased food intake and body weight gain would be considered an undesirable effect if they were to occur in human infants. The NOAEL in the evaluated 3-week neonatal pig study was 0.3% (reported to be equal to 847 mg/kg bw per day) [1].

At the present meeting, the Committee evaluated an amended report [2] that contained an updated statistical analysis of the previously evaluated neonatal pig study [1] and an additional 3-week neonatal pig study [3].

The reanalysis [2] of the previously evaluated 3-week neonatal pig study (six of each sex per dose), which tested pectin at target concentrations of 0.05%, 0.3% and 1% (500, 3000 and 10 000 mg/L) in the milk replacer, proposed that growth data from both sexes could be analysed together. It confirmed that there were no growth effects at 0.05% or 0.3% pectin relative to the control group and that pectin at the highest dose of 1% did not significantly affect consumption of

the milk replacer, but did significantly decrease body weight and feed conversion efficiency in pigs, irrespective of sex. The reanalysis confirmed the Committee's previous conclusion that the NOAEL of pectin in this study is 0.3%. The dose levels for this study were recalculated using measured concentrations of pectin of 458, 3700 and 13 300 mg/L, instead of the target concentrations, to calculate dose levels of 131, 1049 and 4015 mg/kg bw per day for males and 130, 1088 and 4123 mg/kg bw per day for females, respectively. The dose levels in the study when the data for the sexes are combined in the reanalysis are 128, 1064 and 4062 mg/kg bw per day for the 0.05%, 0.3% and 1% groups, respectively. However, the Committee noted that it is JECFA practice to calculate dose levels separately for males and females and to base the NOAEL on the lower of these values, and the NOAEL from this study is therefore 1049 mg/kg bw per day.

In a new study focusing on growth and nutrient digestibility [3], neonatal pigs (six of each sex per dose) were administered pectin in milk replacer as their sole source of nutrition for 3 weeks at a target concentration of 0.2% or 1% (equal to 704 and 4461 mg/kg bw per day, respectively, for males and females combined). No differences between the control and the 0.2% group were observed in any aspect of growth at any time, including average daily milk replacer consumption, daily body weight, average daily body weight gain, feed conversion efficiency and final body weight. In contrast, consumption of milk replacer and growth were significantly reduced in the 1% pectin group. The reduced body weight gain in the 1% pectin group was associated with both lower milk replacer consumption and reduced nutrient digestibility. The Committee concluded that the reduced milk replacer consumption observed in neonatal pigs in both studies at a dose level of 1% pectin in milk replacer was likely due to delayed gastric emptying and/or prolonged gut transit resulting from consumption of the highly viscous 1% pectin diet. The NOAEL for this study was 0.2% pectin (equal to 704 mg/kg bw per day for males and females combined).

Observations in humans

Human studies previously evaluated by the Committee at the seventy-ninth meeting indicated that pectin was well tolerated by preterm infants at a concentration of 0.085% and that pAOS was well tolerated in infants in four studies with pAOS concentrations up to 0.2% in formula.

Assessment of dietary exposure

The maximum proposed use level for pectin in infant formula is 2000 mg/L.

Infant formula consumption estimates were derived from mean 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 per kilogram body weight 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 needs to the volume of formula ingested daily.

Dietary exposure to pectin from its use at the proposed use level in infant formula ranges from 120 to 360 mg/kg bw per day for infants aged 0–12 weeks, whereas infants with high (95th percentile) energy intakes may reach an exposure level of 440 mg/kg bw per day.

Evaluation

The Committee previously assigned a group ADI "not specified" to pectin and amidated pectins, but this group ADI 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. Therefore, special considerations are required for this age group on a case-by-case basis, and toxicological testing strategies for additives to be used in infant formulas require different approaches, including studies involving exposure of very young animals.

The Committee previously concluded that estimated exposure to pectin in infant formula at the then proposed use level (0.5%) was in the region of the NOAEL of 847 mg/kg bw per day in a neonatal pig study and close to the LOAEL of 3013 mg/kg bw per day, based on decreased feed intake and body weight gain, which was of concern. The newly submitted data evaluated at the present meeting confirm these effects and indicate that they are likely due to delayed gastric emptying and/or prolonged gut transit resulting from the viscosity of the material. The re-evaluation of the dose levels using measured concentrations of pectin in milk replacer rather than target concentrations also indicates a slightly higher NOAEL of 1049 mg/kg bw per day. Although the NOAEL in the study by Dilger [3] is lower than that of the MPI Research Inc. [2] study, the Committee noted that this is because of the difference in dose spacing and identified the critical NOAEL as 1049 mg/kg bw per day.

At the new maximum proposed use level of 0.2%, the estimated exposure of infants 0–12 weeks of age would be up to 360 and 440 mg/kg bw per day at mean and high consumption. The MOEs for average and high consumers are 2.9 and 2.4, respectively, when compared with the NOAEL of 1049 mg/kg bw per day.

The Committee noted that the MOEs calculated at the present meeting are within the range of 1–10, which could be interpreted as indicating low risk for the health of infants aged 0–12 weeks consuming a food additive in infant

formula, subject to a number of considerations related to the toxicological point of departure and the exposure assessment (Annex 1, reference *220*). Relevant considerations in relation to pectin are as follows:

- The toxicity of pectin is low.
- The NOAEL is derived from studies in neonatal pigs, which are considered a relevant animal model.
- The adverse effects in the neonatal pig study are likely to be related to the viscosity of pectin at the concentration of 1%.
- Clinical studies provide support for the tolerance of infants to pectin at concentrations up to 0.2%.
- The exposure estimates are conservative.

Overall, the Committee concluded that the MOEs calculated for the use of pectin at 0.2% in infant formula indicate low risk for the health of infants and therefore are not of concern. The Committee recognizes that there is variability in medical conditions among infants requiring formula for special medical purposes and that these infants would normally be under medical supervision.

An addendum to the monograph was prepared.

The Committee at its seventy-first meeting (Annex 1, reference *196*) 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 2.3.3. At the present meeting, the specifications for pectin were revised to lower the limit for lead from 5 to 2 mg/kg for general use, to introduce a limit for lead of 0.5 mg/kg for use in infant formula and to update the method descriptions for the determination of lead and sample preparation for residual solvents.

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3.1.6 Quinoline Yellow

Explanation

Quinoline Yellow (INS No. 104) is a synthetic food colour. It was previously evaluated by the Committee at its eighth, thirteenth, eighteenth, nineteenth, twenty-second, twenty-fifth, twenty-eighth and seventy-fourth meetings (Annex 1, references *8*, *19*, *35*, *38*, *47*, *56*, *66* and *205*).

At its thirteenth meeting, the Committee established a temporary ADI of 0–1 mg/kg bw, based on a no-observed-effect level (NOEL) of 500 mg/kg bw per day in a long-term feeding study in rats. The ADI was temporary because of the absence of suitable information on the metabolism of Quinoline Yellow and a long-term feeding study in another mammalian species. At its eighteenth meeting, the Committee considered a second long-term feeding study in rats and established a temporary ADI of 0–0.5 mg/kg bw, based on the absence of any adverse effects at the highest tested dose of 50 mg/kg bw per day. The Committee reiterated its requirement for a multigeneration reproduction study that was in progress, more information on metabolism and a long-term feeding study in another species.

At its twenty-second meeting, the Committee reviewed a three-generation reproduction study in rats but did not amend the temporary ADI. At its twenty-fifth meeting, the Committee was advised that two major studies were nearing completion and decided to extend the temporary ADI that it had established at its eighteenth meeting until the twenty-eighth meeting.

At the twenty-eighth meeting, the Committee reviewed new data on metabolism and a long-term repeated-dose study in mice that had been exposed to Quinoline Yellow in utero, through lactation and for the next 21–23 months. The Committee established an ADI of 0–10 mg/kg bw, based on a NOEL of 10 000 mg/kg in the diet (equivalent to 1500 mg/kg bw per day) in the long-term study in mice.

At its seventy-fourth meeting, the Committee based its evaluation on data previously reviewed together with published information that had become available since the twenty-eighth meeting. The Committee was aware of unpublished long-term studies in mice and rats with in utero exposure to what was thought, at the time, to be Quinoline Yellow; these studies had been completed by Biodynamics Laboratories in the early 1980s, but had not been submitted for evaluation. The Committee noted that these studies might have an effect on the ADI, so it withdrew the previously established ADI of 0–10 mg/kg bw and established a temporary ADI of 0–5 mg/kg bw, incorporating an additional 2-fold uncertainty factor, pending submission of the Biodynamics Laboratories studies by the end of 2013.

Following a public call for data, the three long-term toxicity and carcinogenicity studies in mice and rats with in utero exposure that had been completed by Biodynamics Laboratories were submitted to the Committee. The test substance in these three studies was found to be D&C Yellow No. 10, not Quinoline Yellow, as had been previously assumed. At the present meeting, it also became clear to the Committee that some of the studies in previous JECFA monographs that had been described as studies on Quinoline Yellow were, in fact, carried out using D&C Yellow No. 10 as the test substance.

At the present meeting, the Committee re-evaluated Quinoline Yellow, taking into consideration the three submitted studies in mice and rats. The Committee also considered other relevant information obtained from a search of the published literature. In addition, the Committee identified, where possible, whether the test substance was Quinoline Yellow or D&C Yellow No. 10 in previously evaluated studies.

Chemical and technical considerations

Quinoline Yellow (INS No. 104) is a synthetic colouring agent that belongs to the class of quinoline dyes. It consists predominantly of sodium salts of disulfonates of 2-(2-quinolyl)-1,3-indandione, with smaller amounts of monosulfonates and trisulfonates. It is allowed as a food colour in the EU, China, Australia and New Zealand.

Quinoline Yellow is manufactured by sulfonating 2-(2-quinolyl)-1,3-indandione. Quinoline Yellow is a yellow-coloured powder or granules and is freely soluble in water, sparingly soluble in ethanol and insoluble in oil. It contains not less than 70% total colouring matters. Of the total colouring matters present, not less than 80% are present as disulfonates, not more than 15% as monosulfonates and not more than 7% as trisulfonates. Subsidiary colouring matters, 2-(2-quinolyl)-1,3-indandione and 2-[2-(6-methyl-quinolyl)]-1,3-indandione, are present at not more than 4 mg/kg. Organic compounds other than colouring matters (total of 2-methylquinoline, 2-methylquinolinesulfonic acid and phthalic acid) are present at not more than 0.5%. Volatile matter and sodium chloride and/or sodium sulfate are the other uncoloured components.

A closely related colour, D&C Yellow No. 10, is an analogous quinoline dye that is not permitted for use as a food colour. It is allowed as a drug and cosmetic colour in the USA, Japan and other countries. It is also manufactured by sulfonating 2-(2-quinolyl)-1,3-indandione, but its sulfonation is more limited. It consists predominantly of sodium salts of the monosulfonates (not less than 75%), with disulfonates not more than 15%. It differs from Quinoline Yellow with a lower proportion of disulfonates, higher proportion of monosulfonates and no trisulfonates.

Quinoline Yellow

Biochemical aspects

The absorption of ingested Quinoline Yellow is between 3% and 4% in rats and dogs, with most being excreted unchanged in faeces. There is evidence that some of the absorbed Quinoline Yellow is excreted in bile. Quinoline Yellow does not accumulate in tissues, and 85–90% of the Quinoline Yellow absorbed from the gastrointestinal tract is excreted unchanged in the urine (Annex 1, reference *206*).

Toxicological studies

No acute or short-term toxicity data were available on Quinoline Yellow. Two-year feeding studies previously reviewed by the Committee suggested the absence of any treatment-related effects at the highest dose administered in the diet to mice and at the only dose tested in rats, equivalent to 1500 and 500 mg/kg bw per day, respectively [1] (Annex 1, reference *206*). The long-term chronic toxicity and carcinogenicity study in mice involving in utero exposure [1] indicated that Quinoline Yellow did not affect reproduction or development.

One in vitro micronucleus test in Chinese hamster V79 cells, reviewed by EFSA [2], was negative, with and without metabolic activation.

Observations in humans

The Committee noted that it had previously considered studies that investigated a possible relationship between hyperactivity in children and the consumption of beverages containing a mixture of food colours, including Quinoline Yellow, and a preservative, sodium benzoate [3, 4]. As concluded previously by the Committee (Annex 1, reference *205*), these studies were of limited value because of inconsistencies in the findings and the use of mixtures of food colours.

There are reports suggesting that asthma or chronic idiopathic urticaria/angio-oedema in humans may be induced by oral exposure to Quinoline Yellow. However, most of these reports are characterized by poorly controlled challenge procedures. Although recent studies performed with better control conditions are available, no conclusion on idiosyncratic responses to Quinoline Yellow could be drawn from the available evidence.

D&C Yellow No. 10

Biochemical aspects

No absorption, distribution, metabolism or excretion data were available on D&C Yellow No. 10.

Toxicological studies

Ninety-day oral toxicity studies in rats and dogs showed no adverse effects at dose levels of 1500 and 750 mg/kg bw per day, respectively [5, 6].

A long-term chronic toxicity and carcinogenicity study in which mice were given D&C Yellow No. 10 continuously in the diet at 0%, 0.1%, 1% or 5% (equivalent to 0, 150, 1500 and 7500 mg/kg bw per day, respectively) for approximately 24 months for males and 23 months for females resulted in no treatment-related adverse effects, including tumours [7]. The Committee concluded that the NOAEL for this study was 7500 mg/kg bw per day, the highest dose tested.

Two long-term chronic toxicity and carcinogenicity studies in rats with an in utero phase, comprising two sequential studies with D&C Yellow No. 10 in the diet at 0%, 0.03%, 0.1% or 0.5% (equivalent to 0, 15, 50 and 250 mg/kg bw per day, respectively) and 0%, 2% or 5% (equivalent to 0, 1000 and 2500 mg/kg bw per day, respectively), revealed no carcinogenic potential of D&C Yellow No. 10. There were slight reductions in body weight and changes in absolute and relative organ weights at the two highest dose levels (2% and 5%) [8, 9]. The Committee concluded that the NOAEL for these two related studies was 0.5% in the diet (equivalent to 250 mg/kg bw per day). No treatment-related effects were noted in a 2-year study in dogs [10], and the Committee concluded that the NOAEL for this study was 0.2% (equivalent to 150 mg/kg bw per day), the highest dose tested.

For D&C Yellow No. 10, in vitro assays for gene mutation, comprising a test in *Salmonella typhimurium* and a test in mouse lymphoma cells, were negative, and an in vivo mouse bone marrow micronucleus test was also negative [11–13].

In a three-generation reproduction study in rats administered D&C Yellow No. 10 in the diet at doses equivalent to 0.5–50.0 mg/kg bw per day, no compound-related effects on parental mortality, body weight, feed consumption, mating, pregnancy or fertility rates, pup survival, pup body weight, reproductive parameters, including numbers of embryos, corpora lutea and resorptions, or necropsy findings were observed. No gross or histological abnormalities were noted in the tissues of rats of the F_{1b} or F_{3a} generation that could be attributed to D&C Yellow No. 10 [14].

In the in utero phase of the long-term chronic toxicity and carcinogenicity studies described above, rats were exposed to D&C Yellow No. 10 in the diet 2 months prior to mating and continuously throughout pregnancy and lactation. Pup viability at birth for the 0.5% dose group was somewhat lower than that of the control group. However, no effect on pup viability was observed in the 2% or 5% dose groups, indicating that it was not a dose-related effect and might be an

incidental finding. In addition, no dose-related effects on the number of pregnant females per group or litter size at birth in all dose groups were noted.

In the developmental toxicity studies performed in rats and rabbits, D&C Yellow No. 10, given as oral gavage doses up to 150 mg/kg bw per day, did not cause maternal toxicity or fetal abnormalities [15, 16].

Assessment of dietary exposure

Estimates of dietary exposure to Quinoline Yellow prepared and published by EFSA and FSANZ were available to the Committee, in addition to published papers for Irish schoolchildren [17–19].

The Committee concluded that EFSA's 95th percentile exposure estimate for European children aged 3–9 years of 0.05–0.29 mg/kg bw per day for a brand-loyal consumer represented the most conservative estimate, based on extensive reported and/or industry use data across all countries and age groups assessed [19]. Available data on estimates of dietary exposure to Quinoline Yellow for children aged 2–16 years who were high consumers based on analytical data from Australia (0.01 mg/kg bw per day, 90th percentile consumers) [18] were of a similar magnitude, but lower than the EFSA estimate. The Committee noted that in the Australian survey, Quinoline Yellow was not detected in flavoured drinks, a food category that was a major contributor to estimated dietary exposure for European populations. Estimates of dietary exposure to Quinoline Yellow for Irish schoolchildren aged 5–17 years based on consumption data at the brand level and concentration data were also in a similar range (0.04–0.08 mg/kg bw per day, 90th percentile consumers), but not as high as the top end of the range reported by EFSA.

The Committee concluded that estimates of dietary exposure to Quinoline Yellow for Europe and Australia utilized the same approach and were comparable and that a range of estimated dietary exposures for children who were high consumers from 0.01 to 0.29 mg/kg bw per day should be used for the safety assessment.

Evaluation

The Committee noted that the method of manufacture for Quinoline Yellow and D&C Yellow No. 10 is the same and that the only major difference between the two colours is in the degree of sulfonation of the components. The specifications for both colours similarly restrict the content of the non-sulfonated impurity, and the specification for Quinoline Yellow has a lower limit for lead than the specification for D&C Yellow No. 10 and additionally has a limit for unsulfonated primary aromatic amines. The Committee therefore concluded that it would be

reasonable to use toxicology data on D&C Yellow No. 10 to support the database for Quinoline Yellow.

The Committee concluded that the existing data on Quinoline Yellow and D&C Yellow No. 10 provide a sufficient basis on which to establish an ADI for Quinoline Yellow. The two related long-term studies on D&C Yellow No. 10 in the rat gave the lowest NOAEL of 0.5% in the diet (equivalent to 250 mg/kg bw per day), based on effects on body weight and organ weights at higher dose levels. Using this NOAEL and an uncertainty factor of 100, the Committee established an ADI of 0–3 mg/kg bw (rounded value) for Quinoline Yellow.

The Committee noted that the range of estimated dietary exposures to Quinoline Yellow for children based on analytical, reported and/or industry use data, including the conservative estimate by EFSA, was below the upper bound of the ADI (0.3–10%). The Committee concluded that dietary exposure to Quinoline Yellow for children and all other age groups does not present a health concern.

A monograph was prepared.

The Committee, at the seventy-fourth meeting, recognized that the specifications for Quinoline Yellow had been inadvertently published as full specifications; the Committee prepared revised tentative specifications and requested additional information.

At the present meeting, based on the information available, the Committee revised the methods for determining lead and zinc, replaced the titanium trichloride assay with assay by spectrophotometry, added the maximum wavelength of absorbance and absorptivity value for the colour dissolved in water, and added HPLC conditions for determining the subsidiary colouring matters and organic compounds other than colouring matters and for assaying the colouring components.

The specifications were revised, and the tentative status was removed.

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3.1.7 Rosemary extract

Explanation

Rosemary extract (INS No. 392) is obtained from ground dried leaves of *Rosmarinus officinalis* L. and has been shown to possess antioxidant properties.

The antioxidant characteristics of rosemary extract are primarily attributed to its phenolic diterpene content – namely, carnosic acid and carnosol. Rosemary also contains several volatile components that contribute to its characteristic flavour. The rosemary extract for use as an antioxidant has a minimum ratio of total content of carnosic acid and carnosol to total volatile components of 15:1.

Following the Twenty-third Session of the Codex Committee on Fats and Oils (CCFO) in 2013 [1], CCFO decided to refer to CCFA its intention to include "rosemary extract" as an antioxidant in the standard for fish oils, noting that it had not yet been included in the GSFA. At the Forty-fifth Session of CCFA in 2013 [2], it was concluded that although rosemary extract had been assigned an INS number (392), it had not yet been evaluated by JECFA.

The Committee evaluated rosemary extract at the present meeting at the request of CCFA.

At the present meeting, the Committee evaluated a number of unpublished toxicological studies submitted by the sponsor. In addition, the Committee reviewed published studies identified in the scientific literature that were of relevance to the safety assessment of rosemary extract.

Chemical and technical considerations

Rosmarinus officinalis L. is a small evergreen shrub, indigenous to Mediterranean Europe, and belongs to the Lamiaceae family. Rosemary extract is obtained from ground dried leaves of *R. officinalis* using food-grade acetone or ethanol. The crude extract is then subjected to filtration, concentration and solvent evaporation, followed by drying and sieving to obtain a fine beige powder of the native rosemary extract. Additional concentration and/or precipitation steps followed by deodorization, decolorization and standardization using food-grade diluents and carriers are included in the downstream processing of the final product for commerce.

The composition of rosemary extract is influenced by the rosemary plant's natural variability, cultivation conditions, treatment of the leaves prior to extraction, and the extraction process itself. The rosemary extract is characterized by the presence of two main phenolic diterpenes – carnosic acid (CAS No. 3650-09-7, molecular formula $C_{20}H_{28}O_4$) and carnosol (CAS No. 5957-80-2, molecular formula $C_{20}H_{26}O_4$), which are major contributors to the antioxidant activity. Rosemary extract also contains several other antioxidants that belong to the classes of phenolic acids, flavonoids, diterpenoids and triterpenes. In addition, rosemary extract contains volatiles, tannins, polyphenols, polysaccharides and lipophilic substances. The key volatile components in rosemary extract are 1,8-cineole (eucalyptol), camphor, borneol, bornyl acetate and verbenone. The product of commerce can be standardized to a total content of carnosic acid and

carnosol of up to 33%, and its use as an antioxidant is differentiated from its use as a flavouring by the identity test.

Biochemical aspects

The disposition of carnosic acid (purity 98% and 91%) in male rats was determined following intravenous and oral gavage administration. In a study by Yan et al. [3], the plasma levels of carnosic acid following oral administration (90 mg/kg bw) revealed an apparent elimination half-life of 962 minutes, which was approximately 14 times longer than the apparent elimination half-life following intravenous administration (68 minutes). This result indicates that the terminal slope in the oral plasma concentration–time curve is not truly representative of the elimination process. It suggests that the rate-limiting step is likely the absorption of carnosic acid from the gastrointestinal tract and not its elimination from plasma. Orally administered carnosic acid (90 mg/kg bw) was detected in stomach, liver and small intestine at maximum concentrations of 1871, 16 and 34 µg/g, respectively, but it was not detected in other tissues with high blood flow, such as heart, kidney and lung [4]. Yan et al. [3] reported that the time to peak concentration (T_{max}) of carnosic acid in plasma following oral dosing was around 126 minutes, and the absolute bioavailability was calculated to be 65%. Doolaege et al. [5] observed a similar T_{max} (137 minutes) and a bioavailability of around 40%. No evidence for enterohepatic circulation of carnosic acid was observed in either pharmacokinetic study [3, 5] following intravenous administration.

Incubation of human and rat liver microsomes with carnosic acid resulted in similar metabolic profiles, providing evidence that carnosic acid undergoes similar biotransformation in the two species [6]. Zuo [4] and Song et al. [6] reported that carnosic acid is extensively metabolized in rats, with four metabolites detected in bile and faeces and an additional 15 detected in urine. Evidence indicates that carnosic acid can be oxidized to carnosol and further metabolized via glucuronidation and methylation reactions [6]. The predominant metabolite of carnosic acid was glucuronidated carnosic acid. Doolaege et al. [5] reported that 15.6 ± 8.2% of carnosic acid administered orally was recovered in the faeces of rats over a 24-hour period post-administration.

To identify the metabolites formed, a commercial rosemary extract (571 mg/kg bw, equivalent to 230 mg/kg bw expressed as carnosic acid) was administered to rats by gavage following a 24-hour fast. These rats had received the same extract in their diet for 2 weeks prior to the gavage administration. Carnosic acid was detected in plasma after 25 minutes, and this was considered to be the T_{max}. The maximum plasma concentration for the main conjugate of carnosic acid, carnosic acid glucuronide, was reported at the last sampling time of 800 minutes. The most abundant metabolites quantified in plasma were the

5,6,7,10-tetrahydro-7-hydroxyrosmariquinone and carnosic acid 12-methyl ether. Nine major metabolites were identified in the liver. Small quantities of carnosic acid 12-methyl ether and carnosic acid (1.9–4.0 µg/g) were detected in brain tissue. A number of metabolites of carnosic acid indicative of both glucuronidation and methylation were identified following the oral administration of a commercial rosemary extract to rats [7]. These results were consistent with the metabolic profile elucidated for carnosic acid following oral administration to rats [6].

In summary, oral bioavailability for carnosic acid has been estimated to be 40–65%, characterized by relatively slow absorption from the gastrointestinal tract. In vitro, similar metabolic profiles of carnosic acid have been observed using human and rat liver microsomes. In vivo, carnosic acid is extensively metabolized by direct glucuronidation and/or methylation reactions, as well as oxidation of carnosic acid to carnosol. Additional metabolites of carnosic acid and carnosol can undergo further glucuronidation, oxidation and/or methylation reactions, with several metabolites identified in liver, urine and faeces of rats.

Hepatic enzyme induction was reported in primary cultures of human hepatocytes following exposure of the cells to carnosic acid, as evidenced by upregulation of CYP2B6 and CYP3A4 mRNA levels in a concentration-dependent manner [8]. In female rats treated with supercritical carbon dioxide extract of rosemary (33% weight per weight [w/w] carnosol plus carnosic acid content) at a dose equal to 195 mg/kg bw per day, total hepatic microsomal P450 content was increased by approximately 1.5-fold compared with controls following a 13-week treatment period; similar minimal increases were observed in levels of hepatic CYP2A, CYP2C11, CYP2E1 and CYP4A activity. No induction of activities associated with CYP1A, CYP2B or CYP3A was noted. This enzyme induction was observed to be reversible following a 4-week treatment-free period [9]. Elevated liver enzyme activity (glutathione *S*-transferase [GST] and quinone reductase) was also observed in mice and rats fed commercial extracts of rosemary in the diet at concentrations of up to 10 000 mg/kg (equivalent to up to 900 and 500 mg/kg bw per day for mice and rats, respectively) for 2–4 weeks [10, 11], but not for carnosol [10].

Toxicological studies

A range of studies on acute toxicity, short-term toxicity and genotoxicity were evaluated in the safety assessment of rosemary extract.

Rosemary extracts and an isolated extract constituent, carnosic acid, have low acute oral toxicity in rats and mice. The oral median lethal dose (LD_{50}) was greater than 2000 mg/kg bw for rosemary extracts administered by gavage to rats [12, 13] and was 7100 mg/kg bw for carnosic acid administered by gavage to mice [14].

Short-term studies (14–90 days) investigating the toxicity of five different solvent extracts of rosemary (acetone, ethanol, deodorized ethanol, supercritical carbon dioxide and hexane-ethanol) administered in the diet were assessed in rats. Rats were administered extracts of rosemary in the diet at dose levels ranging between 26 and 400 mg/kg bw per day. Depending on the type of extract, the carnosic acid and carnosol content ranged from 5% to 33%, and the rats were exposed to carnosol and carnosic acid at a dose range of 3–69 mg/kg bw per day [9, 15–18].

In the 90-day studies conducted with solvent extracts of rosemary, a common observation was an increase in relative liver weight in treated animals compared with controls (10–21%). These observations in the liver were also associated with centrilobular hypertrophy, cytoplasmic characteristics of increased glycogen storage and increases in smooth endoplasmic reticulum. As no changes in clinical chemistry or any morphological features of liver damage were observed in the same studies, the Committee concluded that the observed hepatic changes are consistent with a common adaptive response of rodent livers and are not adverse [19]. Slight bile duct hyperplasia was observed in high-dose rats after 4 weeks of exposure to the hexane-ethanol extract [18]. The bile duct hyperplasia decreased with increasing duration of exposure and was not associated with any increase in blood bilirubin or enzyme markers indicative of biliary obstruction or hepatocyte damage. The Committee concluded that the observed bile duct hyperplasia in high-dose rats was not adverse.

NOAELs for each of these short-term studies were identified as the highest dose tested on the basis of an absence of adverse effects. The highest NOAEL expressed as carnosic acid plus carnosol in the 90-day studies was 64 mg/kg bw per day.

No chronic toxicity studies conducted with extracts of rosemary were available.

The genotoxicity potential was assessed for the supercritical carbon dioxide, ethanol and hexane-ethanol extracts of rosemary and the two primary constituents, carnosic acid and carnosol, in prokaryotic and eukaryotic test systems in vitro [13, 20–23] and in two in vivo assays [24, 25]. The results did not indicate a genotoxic concern. No studies evaluating the genotoxicity potential of acetone extract of rosemary were identified; however, genotoxicity data for the acetone extract of rosemary were not considered necessary, based on the absence of significant differences noted in the compositions of the solvent-based extracts or in the toxicological observations from the short-term toxicity studies.

Studies examining the potential reproductive or developmental toxicity of extracts of rosemary have not been conducted with any of the five solvent-based extracts. In a reproductive study conducted with a hydro-alcoholic (70% ethanol:30% water) extract of rosemary [26], significant effects related to reduced

reproductive organ weights and sperm parameters were observed in male rats at a rosemary extract dose of 500 mg/kg bw per day in water. The relevance of this reproductive study to the current assessment was questioned by the Committee, as none of the commercial extracts used in the short-term feeding studies is soluble in water, and significant compositional differences between aqueous and solvent-based extracts of rosemary would be expected [27]. In the short-term toxicity studies conducted for each of the solvent-based extracts, no treatment-related adverse effects in reproductive organs of male or female rats were observed at doses up to 180–400 mg/kg bw per day, the highest doses tested, equivalent to approximately 20–64 mg/kg bw per day expressed as carnosol and carnosic acid, depending on the type of extract [9, 16–18]. In a developmental toxicity study [28], a water-based rosemary extract at a dose of 130 mg/kg bw per day caused no significant effects on preimplantation or post-implantation loss or on the number of variations or malformations in term fetuses.

Observations in humans

Published studies in which humans were administered commercial extracts of rosemary (extraction method not provided) reported that consumption of a single dose (0.32 mg/kg bw expressed as carnosol plus carnosic acid) [29] or repeated doses (0.13 mg/kg bw per day expressed as carnosol plus carnosic acid) for 21 days [30] was not associated with adverse effects in young healthy individuals. In addition, rosemary (and its constituents) has a long history of consumption as part of the normal human diet as a seasoning.

Assessment of dietary exposure

Estimates of dietary exposure to rosemary extract as an antioxidant for populations in Europe and the USA were available to the Committee from the sponsor and EFSA [13, 31]. The Committee noted that the estimates for these two population groups are considered to be conservative estimates of dietary exposure, in that it is assumed that all food products within a food category contain rosemary extract at the maximum permitted level of use. The highest estimates of dietary exposure to rosemary extract for consumers in European populations were observed for toddlers (0.09–0.44 mg/kg bw per day) at the mean level of exposure and for children aged 4–9 years (0.25–0.81 mg/kg bw per day) at the 95th percentile exposure (expressed as carnosol plus carnosic acid). The highest estimates of dietary exposure to rosemary extract for consumers in the population in the USA were for infants and young children aged 0–3 years, using food consumption data from the United States National Health and Nutrition Examination Surveys (NHANES) 2011–2012 in conjunction with the EU maximum permitted level of use for rosemary extract; the estimates (expressed as carnosol plus carnosic acid)

were 0.18 mg/kg bw per day at the mean level of exposure and 0.40 mg/kg bw per day at the 90th percentile exposure. Two main contributors to dietary exposure for European populations were fine bakery wares (6.5–57.8%) and processed meat (8.1–63.4%) across all age groups. No information on the main contributing food groups in the USA was reported. Rosemary is also consumed as a seasoning, but use levels vary dramatically according to taste, and the Committee concluded that this contribution need not be further considered because of the conservative nature of the assumptions applied in the assessments for rosemary extract. Therefore, the Committee concluded that the overall dietary exposure estimates for high consumers in all age groups (95th percentile exposure in the EU and 90th percentile exposure in the USA) ranging from 0.09 to 0.81 mg/kg bw per day should be used for the safety assessment of rosemary extract.

Evaluation

The Committee concluded that there are sufficient data to establish an ADI for rosemary extract prepared according to the specifications established at this meeting.

The Committee established a temporary ADI of 0–0.3 mg/kg bw for rosemary extract, expressed as carnosic acid plus carnosol, on the basis of a NOAEL of 64 mg/kg bw per day, expressed as carnosic acid plus carnosol, the highest dose tested in a short-term toxicity study in rats, with application of a 200-fold uncertainty factor. The overall uncertainty factor of 200 incorporates a factor of 2 to account for the temporary designation of the ADI. The Committee made the ADI temporary pending the submission of studies to elucidate the potential developmental and reproductive toxicity of the rosemary extract under consideration. An additional uncertainty factor to account for the lack of a chronic toxicity study was not considered necessary based on the absence of adverse effects in the short-term toxicity studies at doses up to and including the highest dose tested. The temporary ADI applies to rosemary extract that meets the specifications prepared at the present meeting. The temporary ADI will be withdrawn if the required data are not provided by the end of 2018.

The Committee noted that the dietary exposure estimates for rosemary extract for high consumers in the European and USA populations of 0.09–0.81 mg/kg bw per day (expressed as carnosic acid plus carnosol) may exceed the upper bound of the temporary ADI by up to 2.7-fold (for young children at the top end of the range of estimated dietary exposures). Based on the conservative nature of the dietary exposure assessments, in which it was assumed that all foods contained rosemary extracts at the maximum use level, the Committee concluded that this exceedance of the temporary ADI does not necessarily represent a safety

concern. The Committee requested that data on typical use levels in foods be provided by the end of 2018 in order to refine the dietary exposure estimates.

A monograph was prepared.

The Committee considered both gas chromatography–mass spectrometry (GC-MS) and gas chromatography–flame ionization detection (GC-FID) methods for the determination of key volatiles of rosemary extract and included the published GC-MS method only. The Committee prepared tentative specifications and requested validation information on the method for determination of residual solvents by the end of 2018.

A Chemical and Technical Assessment was prepared for rosemary extract.

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3.1.8 Steviol glycosides

Explanation

Steviol glycosides are natural constituents of the plant *Stevia rebaudiana* Bertoni, which belongs to the Compositae family. Stevioside and rebaudioside A are the glycosides that have been of principal interest for their sweetening properties. Several other steviol glycosides, including rebaudioside D and rebaudioside M, are of recent interest.

At its fifty-first meeting, the Committee evaluated toxicological data on stevioside and the aglycone steviol (Annex 1, reference *137*) and specified that further information was needed. Based on new data and information, at its

sixty-third meeting (Annex 1, reference *173*), the Committee determined that the commercial material should be known as "steviol glycosides" and established tentative specifications for material containing not less than 95% of the total of four specified glycosylated derivatives of steviol (i.e. stevioside, rebaudioside A, rebaudioside C and dulcoside A). Additionally, the sum of stevioside and rebaudioside A content was specified at not less than 70% of the four steviol glycosides.

Also at its sixty-third meeting, the Committee reviewed additional biochemical and toxicological data on the major steviol glycosides and on the aglycone steviol. A temporary ADI of 0–2 mg/kg bw for steviol glycosides, expressed as steviol, was established on the basis of the NOEL of 2.5% stevioside in the diet, equal to 970 mg/kg bw per day, or 383 mg/kg bw per day expressed as steviol, in a 2-year study in rats and the application of an uncertainty factor of 200. The overall uncertainty factor of 200 incorporated a factor of 2 related to the need for further information on the pharmacological effects of steviol glycosides in humans. The Committee specified the need for studies involving repeated exposure of normotensive and hypotensive individuals and patients with type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetes to dietary and therapeutic doses. This was because the evidence available at the time was inadequate to assess whether the pharmacological effects of steviol glycosides would also occur at estimated dietary exposure levels.

At its sixty-eighth meeting (Annex 1, reference *184*), the Committee extended the temporary ADI of 0–2 mg/kg bw for steviol glycosides, expressed as steviol, pending submission of the results of ongoing clinical studies.

At the sixty-ninth meeting (Annex 1, reference *190*), the Committee considered new studies, which included four toxicological studies with rebaudioside A in experimental animals and clinical trials on the effects of steviol glycosides on blood pressure in healthy volunteers with normal or low-normal blood pressure and on glucose homeostasis in men and women with type 2 diabetes mellitus. The results of the new studies showed no adverse effects of steviol glycosides when taken at doses of about 4 mg/kg bw per day, expressed as steviol, for up to 16 weeks by individuals with type 2 diabetes mellitus and individuals with normal or low-normal blood pressure for 4 weeks. The Committee concluded that the new data were sufficient to allow the additional uncertainty factor of 2 and the temporary designation to be removed and established an ADI for steviol glycosides of 0–4 mg/kg bw, expressed as steviol.

At the present meeting, the Committee considered information that had become available since the sixty-ninth meeting. This information was provided in two submissions. The first submission included information to support the safety of rebaudioside A produced by fermentation in a strain of the yeast *Yarrowia lipolytica*, which was genetically engineered to express the steviol

glycoside metabolic pathway of *Stevia rebaudiana*. This submission included a 90-day study of toxicity in rats and two in vitro studies of genotoxicity on this rebaudioside A product. The second submission included in vitro studies investigating the hydrolysis by colonic microflora of several steviol glycosides, including rebaudiosides A to F and rebaudioside M, new toxicokinetic studies on stevioside in humans and rats, and other published studies that had become available since the sixty-ninth meeting, and requested changes to the specifications to expand the definition of steviol glycosides. A literature search was conducted by a sponsor, and relevant publications were submitted.

Chemical and technical considerations

Steviol glycosides are a group of compounds naturally occurring in the plant *Stevia rebaudiana* Bertoni that share a similar molecular structure, where different sugar moieties are attached to a steviol backbone (an *ent*-kaurene-type diterpene). There are two methods of manufacture for products containing steviol glycosides.

Steviol Glycosides from *Stevia rebaudiana* Bertoni are produced from the crushed leaves of the stevia plant, *Stevia rebaudiana* Bertoni, by extraction with hot water and recovered from the aqueous extract using only alcohols and ion exchange resins for the isolation and purification of the product. The commercial product contains not less than 95% of total steviol glycosides (on a dried basis) determined as the sum of all compounds containing a steviol backbone conjugated to any number or combination of the principal sugar moieties in any of the orientations occurring in the leaves of *Stevia rebaudiana* Bertoni, including glucose, rhamnose, xylose, fructose and deoxyglucose. The steviol glycosides composition of the product varies depending upon the composition within the leaves of the *Stevia rebaudiana* Bertoni plant, which is influenced by both soil and climate, and the extraction and purification processes that are used during the manufacturing.

Rebaudioside A from Multiple Gene Donors Expressed in *Yarrowia lipolytica* is produced by fermentation of a genetically modified strain of *Yarrowia lipolytica* to express the *Stevia rebaudiana* Bertoni metabolic pathway. It is composed of at least 95% (on the anhydrous basis) of rebaudioside A (13-[(2-*O*-β-D-glucopyranosyl-3-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)-oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester; CAS No. 58543-16-1; chemical formula $C_{44}H_{70}O_{23}$), with minor amounts of other steviol glycosides.

Biochemical aspects

Studies previously evaluated by the Committee showed that stevioside and rebaudioside A are poorly absorbed following oral administration, but they

are hydrolysed by intestinal microflora to steviol, which is well absorbed. After absorption, steviol is metabolized to several metabolites, with steviol glucuronide as the major metabolite. In humans, steviol glucuronide is excreted in the urine, but in rats, steviol glucuronide is excreted in the bile and deconjugated in the lower intestine, before elimination as steviol in the faeces.

For the present meeting, the Committee considered an oral absorption study in humans administered stevioside in water and oral absorption studies in rats administered stevioside by gavage and rebaudioside A and rebaudioside D in the diet. The Committee also considered new in vitro studies in which various steviol glycosides were incubated with colonic microflora from both rats and humans.

The results of the new incubation studies were consistent with the findings of similar studies previously evaluated by the Committee. In vitro incubation studies on rebaudiosides A to F, rebaudioside M, steviolbioside, dulcoside A and fructosylated rebaudioside A showed that these compounds are hydrolysed at varying rates to steviol by colonic microflora from both rats and humans [1–5].

In a 28-day rat study, administration of rebaudioside A at a dietary concentration giving a dose of approximately 2000 mg/kg bw per day resulted in maximum plasma concentrations of rebaudioside A, steviol and steviol glucuronide of 1.5, 12 and 50 μg/mL (1.6, 38 and 98 μmol/L), respectively. An analogous study of rebaudioside D in the diet gave maximum plasma concentrations of rebaudioside D, steviol and steviol glucuronide of 0.2, 7 and 19 μg/mL (0.2, 22 and 37 μmol/L), respectively [1].

Oral studies in rats and humans administered a single dose of stevioside resulted in systemic exposure to steviol and substantially greater systemic exposure to its major metabolite, steviol glucuronide [6]. At a stevioside dose of 40 mg/kg bw, toxicokinetic parameters were similar in male and female rats. The area under the plasma concentration–time curve (AUC) for steviol in male and female rats was 581 and 605 h·ng/mL, respectively. The AUC for steviol glucuronide in male and female rats was 2310 and 2500 h·ng/mL, respectively. Peak concentration (C_{max}) values for steviol were 76 ng/mL (males) and 87 ng/mL (females), and for steviol glucuronide were 160 ng/mL (males) and 200 ng/mL (females). The time to peak plasma concentration (T_{max}) of steviol and steviol glucuronide was 4 and 6 hours (males) and 6 and 4 hours (females), respectively. At a stevioside dose of 1000 mg/kg bw, AUC values for steviol and steviol glucuronide were 2.8 and 2.7 times greater, respectively, in females than in males. A possible explanation for these findings is that at the high dose of 1000 mg/kg bw, conversion of stevioside to steviol by intestinal microflora was less efficient in male rats than in female rats, resulting in less steviol available for systemic absorption.

In the human study in which 10 males were administered stevioside at the lower dose level used in the above rat study (40 mg/kg bw), AUC values for

steviol and steviol glucuronide were 1630 and 136 000 h·ng/mL, respectively. C_{max} and T_{max} values for steviol and steviol glucuronide were 77 and 4470 ng/mL and 19 and 22 hours, respectively. All subjects were required to avoid low-calorie and no-calorie foods or beverages containing non-nutritive sweeteners prior to the trial; however, there were six subjects with detectable steviol glucuronide in pre-dosing plasma samples [6].

Toxicological studies

Short-term studies of toxicity were available for stevioside in mice and rats and for rebaudiosides A and D in rats. In a 90-day rat study with stevioside, treatment-related effects on a number of clinical chemistry and haematology parameters and weights of several organs were reported at a dose of 1500 mg/kg bw per day, the highest dose tested [7]. The findings from this study are not consistent with the results of previous toxicity studies on stevioside or other steviol glycosides. Critical reviews of the study were subsequently published, noting a number of potential flaws and inconsistencies [8, 9]. In response, the lead study author stated that a follow-up study would be conducted [10]; however, no subsequent study has been found in the published literature.

No treatment-related adverse effects were reported in the two new repeated-dose toxicity studies evaluated by the Committee. In these studies, doses of stevioside were up to 11 000 mg/kg bw per day in mice, and doses of rebaudioside A or D were up to 2000 mg/kg bw per day in rats [1, 11]. A 90-day toxicity study on rebaudioside A produced in yeast resulted in no treatment-related adverse effects at dose levels up to 2000 mg/kg bw per day, the highest dose tested. Genotoxicity studies on this rebaudioside A product and rebaudioside A isolated from *S. rebaudiana* were negative [12, 13].

Observations in humans

No new human studies were available.

Assessment of dietary exposure

Dietary exposure to steviol glycosides was evaluated using sugar substitution methods and by assessing submitted estimates of dietary exposure to steviol glycosides that had been prepared and published by EFSA [14, 15] and FSANZ [16], in addition to published papers on dietary exposure for the Korean population [17] and information from industry. Dietary exposure results are presented as steviol equivalents per kilogram body weight per day and, if necessary, converted from published values to steviol equivalents based on a conversion factor of 0.4 from stevioside; for a mixture of steviol glycosides, a

range of conversion factors from 0.2 to 0.7 derived from the molecular weights of individual steviol glycosides was used.

Potential dietary exposures to steviol glycosides may be predicted by substituting all sugar in the diet with the intense sweetener using the 17 WHO cluster diets and converting the per capita sugar category amount, assuming a sucrose equivalence of 200 [18]. Predicted dietary exposures to steviol glycosides ranged from 0.5 to 7.2 mg/kg bw per day, expressed as steviol equivalents, across different areas of the world represented by the 17 diets, using the range of sucrose equivalence factors for different mixtures. Similar results were found by substituting total sugar intakes reported from national nutrition surveys for populations in the USA and Australia with steviol glycosides (1.4–6.6 mg/kg bw per day, expressed as steviol equivalents, using the range of sucrose equivalence factors for different mixtures) [19, 20]. Alternatively, a new sweetener can be considered to replace other known intense sweeteners, adjusting for relative sweetness; in a study previously considered by the Committee, predicted dietary exposure to rebaudioside A ranged from 0.4 to 1.7 mg/kg bw per day [21]. The Committee noted that permitted uses of intense sweeteners have since been extended in many regulations. The Committee considered that sugar substitution methods were generally overestimates of dietary exposure, as not all sugar in food products would be replaced by intense sweeteners, and a number of intense sweeteners are used in the marketplace [22, 23].

The Committee concluded that the 2014 and 2015 EFSA predictions of maximum dietary exposure for high consumers for European toddlers aged 12–35 months (95th percentile) of 2.0–4.3 mg/kg bw per day represented the most conservative estimate for European populations based on European maximum permitted levels of use. The 2011 FSANZ predictions of 4.4 mg/kg bw per day, expressed as steviol equivalents, for Australian children aged 2–6 years and 4.0 mg/kg bw per day, expressed as steviol equivalents, for New Zealand children aged 5–14 years were in a similar range (90th percentile, brand-loyal consumer).

Use of the GSFA maximum use levels for steviol glycosides instead of the EU or Australia/New Zealand maximum permitted levels to predict dietary exposures was considered likely to result in a similar outcome, as the maximum levels for the food categories making a major contribution to dietary exposure (flavoured drinks, breakfast cereals, flavoured and/or fermented dairy-based drinks, fermented milk products, processed fruit and vegetables) were similar in most cases. Estimates of dietary exposure only to stevioside based on analytical data for high consumers in the Republic of Korea aged 1 year and over were of a similar magnitude, but lower than the EFSA or FSANZ estimates (0.8–1.4 mg/kg bw per day, 95th percentile consumers).

The Committee concluded that predictions of maximum dietary exposure to steviol glycosides for Europe, Australia and New Zealand based

on detailed food consumption data and maximum use levels utilized the same approach and were comparable. The Committee concluded that predicted dietary exposures for children ranging from 4.0 to 4.4 mg/kg bw per day, expressed as steviol equivalents, should be used for the safety assessment.

Evaluation

The results of new short-term toxicity studies on steviol glycosides, including rebaudioside A produced in yeast, indicated a lack of treatment-related adverse effects, consistent with the results of previous short-term toxicity studies.

Based on the new toxicokinetic studies on stevioside in rats and humans, one of the submissions proposed to use the human:rat C_{max} or AUC ratio for steviol as a chemical-specific adjustment factor instead of the default uncertainty factor of 4.0 that is used to account for interspecies differences in toxicokinetics when deriving health-based guidance values [24]. For the same stevioside oral dose (40 mg/kg bw), the human:rat AUC ratios for steviol and steviol glucuronide were approximately 2.8 and 59, respectively. The Committee noted that six of the 10 human subjects had detectable steviol glucuronide in pre-dosing plasma samples, which confounds the interpretation of the study.

The Committee concluded that this human toxicokinetic study on a small number of males does not provide a reliable estimate of the variability in toxicokinetics, especially the conversion of steviol glycosides to steviol, in the human population. Therefore, the study cannot be used to justify the use of a chemical-specific adjustment factor to derive an ADI for steviol glycosides. The current ADI of 0–4 mg/kg bw, expressed as steviol, was confirmed. The Committee confirmed that rebaudioside A from multiple gene donors expressed in *Yarrowia lipolytica* is included in the current ADI of 0–4 mg/kg bw, expressed as steviol.

The Committee noted that the predicted maximum dietary exposure to steviol glycosides of 4.0–4.4 mg/kg bw per day for young children who were high consumers exceeded the upper bound of the ADI (100–110%), but for other age groups, the ADI was not exceeded. Considering the conservative nature of the dietary exposure estimate, based on maximum use levels applied to all food consumed from categories with permissions for use in the countries assessed, it is not likely to present a health concern for any age group.

An addendum to the monograph was prepared.

The Committee prepared a new specifications monograph (Rebaudioside A from Multiple Gene Donors Expressed in *Yarrowia lipolytica*) for the yeast-derived product, recognizing that it was manufactured by a distinctly different, biosynthetic process compared with stevia leaf–derived products.

New tentative specifications for steviol glycosides were established, including a new title name (Steviol Glycosides from *Stevia rebaudiana* Bertoni) to reflect the separation of specifications by source material. The Definition and Assay specification was expanded from nine named leaf-derived steviol glycosides to include any mixture of steviol glycoside compounds derived from *Stevia rebaudiana* Bertoni, provided that the total percentage of steviol glycosides is not less than 95%. This was based on information and data provided that products manufactured by methods consistent with the definition contain additional steviol glycosides beyond the nine named compounds produced and in different ratios, and information provided on more than 30 steviol glycosides identified in stevia leaf extracts. New proposed procedures for method of assay to determine the greater than 95% total steviol glycosides specification were discussed by the Committee and deemed insufficient to revise the method at the current meeting. In order to be able to remove the tentative designation from the specifications, the following further information is required by 31 December 2017:

- method of assay to replace the existing method and including as many steviol glycosides as possible (at least those listed in Appendix 1 of the specifications) in steviol glycoside mixtures, along with supporting validation information and chromatograms;
- analytical results from a minimum of five batches for commercial samples, including supporting chromatograms.

A Chemical and Technical Assessment was prepared.

The Committee concluded that it was not necessary to make the ADI temporary because the requested information to complete the specifications refers only to an update of the method and has no safety implications.

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3.1.9 Tartrazine

Explanation

Tartrazine (INS No. 102) is an azo dye used as a synthetic food colour. The Committee previously evaluated tartrazine at its eighth meeting in 1964 (Annex 1, reference *8*), when an ADI of 0–7.5 mg/kg bw was established, based on a NOAEL equivalent to 750 mg/kg bw per day, derived from a chronic toxicity study in rats (as cited in [1], as the JECFA monograph for the eighth meeting is no longer available).

At the present meeting, the Committee re-evaluated tartrazine at the request of the Forty-seventh Session of CCFA [2]. The Committee considered submitted studies as well as relevant information obtained from a search of the published literature.

Chemical and technical considerations

Tartrazine (INS No. 102) is a synthetic colouring agent that belongs to the class of monoazo dyes. It is allowed as a food colour in the EU, Japan, the USA and other regions. It is used for colouring beverages, frozen treats, powder mixes, gelatine products, candies, icings, jellies, spices, dressings, sauces, baked goods and dairy products.

Tartrazine consists mainly of trisodium 5-hydroxy-1-(4-sulfonatophenyl)-4-(4-sulfonatophenylazo)-*H*-pyrazole-3-carboxylate and subsidiary colouring matters, together with sodium chloride and/or sodium sulfate as the principal uncoloured components. It is manufactured by coupling diazotized 4-aminobenzenesulfonic acid with 4,5-dihydro-5-oxo-1-(4-sulfophenyl)-1*H*-pyrazole-3-carboxylic acid or with the methyl ester, the ethyl ester or a salt of this carboxylic acid. It may also be manufactured by condensing phenylhydrazine-4-sulfonic acid with dioxosuccinic acid or oxalacetic acid derivatives. The resulting dye is purified and isolated as the sodium salt. Specified impurities include uncombined starting materials, subsidiary colouring matters related to the primary dye component, lead and unsulfonated primary aromatic amines.

Biochemical aspects

Additional data on metabolism and excretion have been reported since the previous evaluation [3–5]. In rats, tartrazine is poorly absorbed and primarily excreted unchanged in the faeces (approximately 90% after 72 hours). Small amounts are broken down by the gut microflora to produce sulfanilic acid and 4-sulfophenylhydrazine, which are excreted in the urine. In bile duct– and urethra-cannulated animals, tartrazine was excreted not intact, but as aminopyrazolone and sulfanilic acid metabolites.

Toxicological studies

Tartrazine is of low acute toxicity, with an LD_{50} value of greater than 2000 mg/kg bw [6].

A number of short-term studies in rats reported significant changes in some blood chemistry parameters indicative of effects on liver and kidney function at relatively low doses (75–500 mg/kg bw per day) [7–13]. The Committee noted that these effects were not reported in long-term studies that used higher dose levels, nor were there any histopathological effects on the liver or kidney in the long-term studies.

A 104-week carcinogenicity study in mice given 0%, 0.5%, 1.5% or 5% tartrazine in the diet showed no effects other than reductions in body weight at various time points in both sexes at 5% in the diet and slight, but statistically significant, increases in feed consumption in males at 5% in the diet [14]. Although the authors considered the NOAEL to be the highest dose tested, the Committee concluded that 1.5% in the diet, equal to 2173 mg/kg bw per day, was the NOAEL for this study, on the basis of a body weight reduction concurrent with an increase in feed consumption at the higher dose in males.

Two separate but concurrent studies in rats given 0%, 0.1%, 1% or 2% in the diet or 0% or 5% in the diet for between 113 and 125 weeks showed decreases in body weight in females at 1% in the diet and in males (12.2% decrease) and females (16.9% decrease) at 5% in the diet, but there were no effects at 2% in the diet [15]. The Committee concluded that 2% in the diet, equal to 984 mg/kg bw per day, was the NOAEL for this study.

During a 2-year study in Fischer 344 rats given tartrazine in the drinking-water at a concentration of 0%, 1% or 2%, statistically significant increases in mesothelioma in the abdominal cavity in males and endometrial stromal polyps in females in the 1% concentration groups were reported. The incidences of these tumours were not dose dependent, and the authors noted that the incidences were within the historical control range for these tumours in this rat strain [16].

Whereas 25 of the 38 available genotoxicity tests are negative, eight in vitro and five in vivo studies have yielded positive results. The relevance of some

in vitro genotoxicity test systems has been questioned due to non-breakage of the azo-linkage and desulfonation of the metabolic products tested. A customized protocol for the reverse mutation assay, using flavin mononucleotide to accelerate the azo-reduction and hamster S9, which has a lower tendency to inactivate the products of azo-reduction, produced negative results [17, 18]. The Committee noted that the majority of in vitro gene mutation studies with tartrazine were negative (13 out of 15) and agreed that the studies by Prival et al. [18] using the modified protocol were more relevant than others yielding positive results. The Committee also noted that the potential for tartrazine to cause point mutations, if any, would be directed towards cells lining the gut during the transit of metabolites prior to their excretion in the faeces.

The question of whether tartrazine may produce effects at the site of contact in the gut has been investigated in vivo. The Committee considered that more weight should be given to the recent, well-conducted study by Pant [19] in the mouse using oral doses up to 2000 mg/kg bw, which showed no evidence of DNA strand breakage in the stomach, colon or liver, contrary to the results of Sasaki et al. [6]. The results of Poul et al. [20], showing an absence of micronucleus formation in colon cells in vivo in the mouse, are also consistent with the results of Pant [19]. The Committee concluded that tartrazine does not cause genotoxicity at sites of contact in the gastrointestinal tract.

Two of the three in vitro chromosome aberration tests reported positive results. The studies by Patterson & Butler [21] and Ishidate et al. [22] reported significant increases in chromosome aberrations, but did not give any information on cytotoxicity. The Committee noted that the study of Pant [19] showed that tartrazine given orally by gavage at doses up to 2000 mg/kg bw per day for 3 days in the mouse did not induce chromosome damage in the bone marrow. The results of Pant [19] are consistent with the earlier findings of Renner [23], who administered single tartrazine doses up to 200 mg/kg bw orally to hamsters.

The possibility of bone marrow chromosomal damage due to longer-term exposure cannot be entirely dismissed, based on the results of Giri et al. [24] in male rats. In this non-standard study, which did not include a positive control, tartrazine was given at 100–1000 mg/kg diet for up to 9 months; a slight (4-fold) increase in the incidence of chromosome breakage was observed.

The Committee concluded that the overall weight of evidence indicates that tartrazine is not genotoxic. The Committee also noted that this conclusion is supported by the lack of carcinogenicity in the long-term mouse study in which tartrazine was given in the diet at doses up to 9735 mg/kg bw per day or in the three long-term studies in rats in which tartrazine was given in the diet or drinking-water at doses up to 3348 mg/kg bw per day.

Reproductive and developmental parameters were assessed in the rat chronic toxicity studies that included an in utero exposure phase. No significant

effects on reproduction or body weights of the offspring were observed [15]. The Committee concluded that 5% in the diet, equal to 2641 mg/kg bw per day, the highest dose tested, was the NOAEL for reproductive end-points in this study.

No reproductive effects were observed in two developmental neurotoxicity studies [25, 26]. Also, no effects on reproductive parameters were observed in several other developmental neurotoxicity studies in rats using a mixture of colours, including tartrazine, as the test substance [27–30].

Two developmental toxicity studies were available in rats, one with dietary administration and one with drinking-water administration of tartrazine during gestation days 0–19; these showed no adverse effects at doses up to 1000 mg/kg bw per day [31, 32].

In the two developmental neurotoxicity studies in mice, some neurobehavioural effects were observed, but these did not show a dose–response relationship; several of the parameters indicated accelerated achievement of developmental milestones, likely related to the observed increase in offspring body weight, which would not be considered adverse [25, 26]. Studies in rats using a mixture of colours, including tartrazine, as the test substance reported some neurobehavioural or neurochemical effects in the treated offspring [27–30, 33]. However, it is not possible to attribute any effects specifically to tartrazine in these mixture studies, and therefore the Committee considered that they were of no significance for this evaluation.

In neurological studies in juvenile mice and rats given tartrazine orally at doses up to 700 mg/kg bw per day for 30 days, some neurobehavioural and neurochemical effects were reported [33, 34]. The Committee noted that only small numbers of animals per dose group were used, and this precluded the use of these studies for this evaluation.

Observations in humans

A number of case reports have been published showing intolerance or hypersensitivity reactions to tartrazine. Although some of these reactions have been shown to be quite severe, their prevalence appears to be very low (0.12% in the general population) [35]. The thorough review by Elhkim et al. [35] concluded that there is a probable risk of intolerance reactions associated with tartrazine at amounts attainable through normal food consumption in a small subset of the population.

In one study, children whose sensitivity to food colours was classified as "suspected" or "uncertain" by their parents were administered six different randomly allocated doses of 0–50 mg tartrazine per day for 21 days. Twenty-four out of 54 children were rated by their parents using a 30-item behavioural inventory as showing a reaction to tartrazine, but no objective measures were

included in the study [36]. The Committee noted that it had previously considered a number of other studies that investigated a possible relationship between hyperactivity in children and the consumption of beverages containing a mixture of food colours, including tartrazine, and a preservative, sodium benzoate [37]. As concluded previously by the Committee (Annex 1, reference *205*), these studies were of limited value because of inconsistencies in the findings and the use of mixtures of food colours.

Assessment of dietary exposure

Submitted dietary exposure information for tartrazine from the EU [41, 42] and the USA [43, 44] and several other published reports were considered by the Committee. The additional information comprises two reports from a colour survey that included a dietary exposure assessment for Australian adults and children from FSANZ [45, 46], an assessment using French data [35] and a report on patterns of dietary exposure to colours for Irish children and teenagers [47]. Additional information on estimated dietary exposures to tartrazine was identified from a literature search for populations from the Hong Kong Special Administrative Region [48], India [49], Indonesia [50], the Republic of Korea [51, 52] and Kuwait. The study on schoolchildren in Kuwait was not further considered by the Committee, as it was not nationally representative.

Estimates of dietary exposures to tartrazine for European children aged 1–10 years who were consumers ranged between 0.2 and 1.9 mg/kg bw per day at the mean and between 0.4 and 7.3 mg/kg bw per day at the 95th percentile, using maximum reported use levels from seven surveys. The estimates of dietary exposure at the 95th percentile for the population in the USA, based on typical reported use levels for 34 GSFA food categories (0.004–0.013 mg/kg bw per day), are underestimates, as they include both eaters and non-eaters of foods that might contain tartrazine.

The dietary exposure estimates at the 90th percentile for the consumer-only population from the USFDA (0.03–0.09 mg/kg bw per day, consumers only, 2 years of age and older) [43] were of the same order of magnitude as those reported by FSANZ [46] for three age groups of Australian children (0.04–0.08 mg/kg bw per day), using a refined model based on analytically determined tartrazine concentrations. The dietary exposure estimates for Hong Kong Special Administrative Region children (0.02–0.14 mg/kg bw per day, mean) and the Korean population (0.05 mg/kg bw per day, 95th percentile) may be correlated with the above estimates, because those concentrations were all analytically determined. The estimates from Indonesia (0.21–0.64 mg/kg bw per day) were 10 times higher than these estimates, primarily because of high consumption of instant noodles and soft drinks. In Indian studies, levels of tartrazine higher

than those permitted have been detected in commodities consumed by children (sugar confectioneries, beverages and ice candy), with the consequence that high estimates of dietary exposure from five age groups ranging from 1.1 to 3.1 mg/kg bw per day were seen.

The Committee concluded that the 95th percentile exposure estimate for European children aged 1–10 years (0.4–7.3 mg/kg bw per day) should be used for the safety assessment of tartrazine, because it represents a broadly applicable, conservative estimate.

Evaluation

In 1964, the Committee established an ADI of 0–7.5 mg/kg bw. New long-term toxicity, genotoxicity and developmental toxicity studies and studies that included reproductive end-points have become available since that time.

The Committee established an ADI of 0–10 mg/kg bw, on the basis of a NOAEL of 984 mg/kg bw per day in a long-term rat study based on reductions in body weight at the higher dose level [15], with application of a 100-fold uncertainty factor. The Committee withdrew the previous ADI of 0–7.5 mg/kg bw per day.

The Committee noted that the dietary exposure estimate for European children aged 1–10 years was below the upper bound of the ADI (4–73%) and concluded that dietary exposure to tartrazine for the general population, including children, does not present a health concern.

An addendum to the monograph was prepared.

Specifications were prepared at the twenty-eighth meeting of the Committee (Annex 1, reference *66*), and metals and arsenic specifications were revised at the fifty-ninth meeting (Annex 1, reference *160*). At the present meeting, the method for the determination of lead was changed from atomic absorption to any method appropriate to the specified level. Updated HPLC conditions were added for determining subsidiary colouring matters and organic compounds other than colouring matters. The method of assay was changed to visible spectrophotometry, and spectrophotometric data were provided for the colour dissolved in water.

The existing specifications were revised, and a Chemical and Technical Assessment was prepared.

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3.1.10 Xanthan gum

Explanation

Xanthan gum (INS No. 415) is currently permitted for use in a wide range of foods and beverages, with technical functions as an emulsifier, foaming agent, stabilizer or thickener. Uses of xanthan gum in foods intended for infants and young children as listed in the GSFA are currently limited to complementary foods for these age groups.

The safety of xanthan gum for use in food was considered previously by the Committee at its eighteenth, twenty-ninth and thirtieth meetings (Annex 1, references *35*, *70* and *73*). At the thirtieth meeting, the Committee established an ADI "not specified", on the basis of an absence of adverse effects in toxicological studies in rats and dogs supported by the absence of adverse effects in studies involving human subjects (Annex 1, references *73* and *74*). At the present meeting, the Committee was asked to evaluate the safety of xanthan gum with respect to its proposed use as a thickener in protein hydrolysate infant formula, follow-on formula and formula for special medical purposes intended for infants (maximum proposed use level 1000 mg/L).

The evaluation of the safety of xanthan gum for use as a thickener in formula considered the results of a number of unpublished study reports provided by the sponsor. In addition to the submitted data, a literature search was conducted. A consolidated monograph was prepared, which included studies from the previously published monograph, new study details from previously evaluated studies, new studies that had become available since the thirtieth meeting and older studies not previously reviewed by the Committee.

Chemical and technical considerations

Xanthan gum is a high-molecular-weight (of the order of 1000 kDa), water-soluble polysaccharide containing D-glucose and D-mannose as the dominant hexose units, along with D-glucuronic acid and pyruvic acid. It is produced by the fermentation of a carbohydrate source in a pure culture of the naturally occurring bacterium, *Xanthomonas campestris*. The fermentation medium contains sources of carbohydrate and nitrogen together with mineral salts. Once the fermentation process is complete, xanthan gum is recovered from the broth by ethanol or isopropanol precipitation in the form of a sodium, calcium or potassium salt. The resulting coagulum is separated, rinsed, pressed, dried and ground as part of downstream processing. Xanthan gum is marketed as a cream-coloured powder and is used as a thickener, stabilizer, emulsifier and foaming agent.

Specifications for xanthan gum were previously established by the Committee at the fifty-third meeting (Annex 1, reference *143*). Xanthan gum used as a thickener in infant formula, follow-on formula and formula for special

medical purposes intended for infants meets the current specifications published in FAO JECFA Monographs 19 (2016).

Biochemical aspects

Results of in vitro studies and studies involving oral administration of xanthan gum to rats show that xanthan gum is largely not digested by the enzymes in the upper gastrointestinal tract and is poorly absorbed [1–3]. Results of in vitro studies indicate that xanthan gum is susceptible to some microbial degradation in the lower gastrointestinal tract [2, 4]. Ingestion of xanthan gum by rats, dogs and human subjects was associated with variable changes in faecal and/or caecal short-chain fatty acid concentrations [5–8].

Toxicological studies

At the thirtieth meeting (Annex 1, references *73* and *74*), the Committee noted that in mice, rats and dogs, xanthan gum exhibited low oral toxicity, with LD_{50} values ranging from greater than 1000 to greater than 45 000 mg/kg bw [1, 9, 10]. In short-term toxicity studies in animals, effects occurred mainly in the intestine at doses above 700 mg/kg bw per day. These included faecal bulking and water binding, with some increases in intestinal tissue mass. At higher doses, reduced nutrient absorption was reported, which explained reduced weight gain and lower liver weights. Other organ weights were unchanged, and no gross morphological or histological abnormalities were reported. In dogs fed a diet containing xanthan gum for 12 weeks, stool softening was observed at doses above 250 mg/kg bw per day, and occasional diarrhoea was seen at 1000 mg/kg bw per day [11].

Several new studies involving short-term dietary administration of xanthan gum to rats and dogs were identified. Edwards & Eastwood [6] reported increases in caecal tissue weight following feeding of xanthan gum in the diet at 5% (2500 mg/kg bw per day) for 4 weeks to male rats. Increased faecal output and increased faecal short-chain fatty acids were also reported in that study. In a 2-week study, Ikegami et al. [12] also noted heavier intestinal tissue weights, as well as small but significant increases in the length of the small and large intestines in rats fed 5% xanthan gum. Rats fed xanthan gum in the diet exhibited increased concentrations of total bile acids and volume of bile, as well as enhanced digestive enzyme activity; no effects on body weight gain were reported. In a 90-day study conducted with a new xanthan gum product in rats, the authors identified a NOAEL of 3301 mg/kg bw per day, the highest dose tested [13]. In two additional studies of shorter duration (up to 10 days) involving dietary administration of xanthan gum (1.2% xanthan gum, equivalent to 300 mg/kg bw per day) to dogs, incorporation of the polysaccharide in the diet affected stool quality (i.e. increased moisture in the stools and softened stools) [3, 8].

In long-term toxicity and carcinogenicity studies previously reviewed by the Committee, xanthan gum was reported to be well tolerated in rats and dogs at doses up to 1000 mg/kg bw per day provided in the diet for 2 years [14]. No increase in tumour incidence was observed [14]. In a three-generation reproductive toxicity study in rats [14], no adverse effects related to reproduction or in utero or postnatal development were reported when rats were given diets providing xanthan gum at doses up to 500 mg/kg bw per day.

Several special studies have become available since the Committee's last evaluation of xanthan gum. In these studies, xanthan gum was found to reduce the postprandial glucose and insulin response [3], increase bile acid secretion and reduce plasma cholesterol and triglyceride levels [7, 15, 16] and reduce serum uric acid concentrations in normal rats and rats with renal dysfunction [17, 18].

In vitro studies on the effects of xanthan gum on the immune system have shown that xanthan gum induces DNA synthesis in mouse splenic B cells and thymocytes as well as polyclonal IgM and IgG antibody responses in B cells [19]. Takeuchi et al. [20] described the production of interleukin 12 and tumour necrosis factor alpha following incubation of two murine macrophage cell lines with xanthan gum. They also reported that splenocytes obtained from xanthan gum–treated mice had greater natural killer cell activity compared with vehicle-treated mice. In addition, the investigators found that oral administration of xanthan gum inhibited transplanted tumour cell growth. These observations indicate biological activity of xanthan gum on exposed cells, but the relevance to humans is not known.

In a study conducted in rats to specifically assess the potential effects of a number of fluid thickeners on absorption of water, no significant differences in the amount of water absorbed following treatment with xanthan gum–containing fluid (compared with pure water) were reported [21].

Special studies in neonatal pigs

New toxicological studies have been conducted with xanthan gum in neonatal pigs. In an initial study [22], xanthan gum was administered in milk replacer to groups of six male and six female neonatal pigs at a dose of 0, 375 or 3750 mg/kg bw per day (dosing concentrations of 0, 750 and 7500 mg/L, respectively) from lactation day 2 for 20 days. In a follow-up study using the same protocol [23] and conducted by the same laboratory within a 2-month period, the neonatal pigs were provided xanthan gum at a dose of 750 mg/kg bw per day (concentration of 1500 mg/L). The two studies were considered together as a single study by the Committee in its evaluation. All animals survived to scheduled necropsy on postnatal day 22. Observations of green discoloured faeces, soft faeces, watery faeces and increased defecation were noted at the dose of 3750 mg/kg bw per day. At the two lower doses, soft and/or watery faeces were also noted, which is an

expected effect of the xanthan gum. Body weight gains of animals at the two lower doses were similar to those of the controls. At the highest dose, terminal body weights of the neonatal pigs were about 40% lower than those of the controls. There were no adverse effects on haematology or coagulation parameters at any dose. Markedly increased absolute and relative weights of the caecum and colon of both sexes were seen at the high dose of 3750 mg/kg bw per day. At the lower doses (375 and 750 mg/kg bw per day), changes in intestinal weights were smaller and not statistically significant. At the highest dose, treatment-related histological changes (primarily goblet cell hypertrophy/hyperplasia) were noted in the large and small intestines and were rated in severity as minimal to moderate. At the two lower doses, similar histological changes were observed in fewer animals and, when present, were considered minimal in severity; these changes were considered by the Committee to be adaptive and non-adverse [24, 25]. Because minimal inflammation of the rectum was observed in treated and control animals, it cannot be attributed to treatment with xanthan gum.

In conclusion, the findings in studies on neonatal pigs show a significant reduction in the tolerability of milk replacer containing xanthan gum at a dose equivalent to approximately 3750 mg/kg bw per day, as evidenced by significantly lower body weights and minimal to moderate histological changes in the gut. The Committee identified the NOAEL for xanthan gum in neonatal pigs as 750 mg/kg bw per day, on the basis of intolerability and histological changes in the intestines observed at 3750 mg/kg bw per day.

Observations in humans

A series of studies involving full-term infants has been conducted to assess growth outcomes and tolerability as well as mineral absorption in infants fed xanthan gum in protein hydrolysate formula.

When infants were fed reconstituted protein hydrolysate formula containing xanthan gum at concentrations up to 1500 mg/L (doses up to 232 mg/kg bw per day) for 1 week, the xanthan gum–containing formula was better tolerated than the same formula without xanthan gum [26]. Relative to the hydrolysate formula without xanthan gum, infants fed formula with xanthan gum displayed decreases in the percentage of watery stools and in the number of stools per day.

In another study designed to determine the potential tolerability of formula differing in carbohydrate source, stabilizers and/or emulsifiers, infants were fed xanthan gum–containing formula (xanthan gum concentration 750 mg/L, dose 120–126 mg/kg bw per day) with or without OSA-modified starch for 20–28 days [27]. The growth outcomes (body weights and body weight gains) among the groups and mean rank stool consistencies were similar. Infants fed

formula with xanthan gum passed significantly fewer stools per day compared with those fed formula without xanthan gum. The authors noted that there were no clinically relevant differences in serious adverse events between the treatment groups with and without xanthan gum, but that the presence of xanthan gum in the formula decreased vomiting. The highest dropout rate related to formula intolerance was reported in the group consuming formula without xanthan gum. The overall conclusion regarding these two studies is that xanthan gum at concentrations of 750–1500 mg/L (doses 120–232 mg/kg bw per day) is well tolerated and does not affect growth characteristics.

In a study spanning up to 112 days, infants received either reconstituted protein hydrolysate formula with xanthan gum (750 mg/L; equivalent to 102 mg/kg bw per day) or an equivalent ready-to-feed formulation without xanthan gum [28]. Parameters evaluated in this study included body weight, food intake, growth outcomes (i.e. body weight gains, length and head circumference) and stool patterns. Feeding of xanthan gum–containing formula for up to 112 days did not adversely affect infant growth or development. The only statistically significant differences reported in this study were related to formula intake and stool production. Infants in the group receiving the ready-to-feed formula displayed greater intakes of the formula and passed more stools per day than did infants receiving the xanthan gum–containing formula. Additionally, parents of infants on the xanthan gum–containing formula responded more frequently that their babies were likely to pass less than one stool per day.

In two special studies ($n = 6$ or 22 infants) examining the potential effects of xanthan gum in formula on mineral absorption (750 and 1500 mg/L), slight decreases in mineral absorption were observed, which did not reach statistical significance [29, 30]. Fractional calcium absorption was lower in the infants fed formula with xanthan gum, but net calcium absorption was similar to that of the infants fed non-xanthan gum–containing formula. With inclusion of xanthan gum in the protein hydrolysate formula, total zinc absorption and net zinc absorption were lower compared with the non-xanthan gum–containing formula. Despite the differences reported in mineral absorption in the studies, no effects on the growth of infants fed xanthan gum–containing formula were reported in these studies or in the 112-day infant growth study [28]. Post-market surveillance data collected by one manufacturer do not indicate any concerns related to growth of infants fed formula containing xanthan gum.

Cases of late-onset necrotizing enterocolitis in newborns (mostly premature) consuming breast milk or formula containing a xanthan gum–based thickener have been reported [31, 32]. The concentrations of xanthan gum in these preparations were not reported. It is not possible to conclude, on the basis of available information, whether there is any causal association with intake of xanthan gum.

Post-marketing surveillance data were collected by one manufacturer over a 5-year period (June 2010 through May 2015) during which distribution of the hydrolysed powder containing xanthan gum (reconstituted at xanthan gum concentrations up to 1000 mg/L) and the ready-to-feed equivalent (without xanthan gum) equalled 105 million litres (providing exposure of a total of 131 million patient treatment days[3] from the manufacturer's products). The data show that consumption of formula containing xanthan gum is not associated with an increased rate of adverse events. The rate for any adverse event was less than one report per 10 000 patient treatment days. Overall, the post-market surveillance data provide additional support for the safe use of xanthan gum in infant formula, including specialized formula consumed by infants with protein allergy [33].

Assessment of dietary exposure

The maximum proposed use level for xanthan gum in infant formula is 1000 mg/L.

Infant formula consumption estimates were derived from mean 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 per kilogram body weight 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 needs to the volume of formula ingested daily.

Dietary exposure to xanthan gum from its use at the maximum proposed use level in infant formula ranges from 60 to 180 mg/kg bw per day in infants aged 0–12 weeks, whereas infants with high (95th percentile) energy intakes may reach an exposure level of 220 mg/kg bw per day.

Evaluation

The Committee previously established an ADI "not specified" for xanthan gum, which does not apply to infants under 12 weeks of age because they might be at risk at lower levels of exposure compared with older age groups. This ADI was based on the absence of toxicity in animal studies, including long-term rat and dog studies in which animals were fed xanthan gum at doses up to 1000 mg/kg bw per day.

[3] One patient treatment day is equivalent to the consumption of 0.8 L of product by an infant in a 24-hour period.

A few additional short-term toxicity and special studies in rats and dogs related to the safety of xanthan gum have become available since the Committee's last evaluation of xanthan gum. Results of these studies confirm the absence of any adverse effects arising from xanthan gum consumption. In clinical studies involving infants, formulas containing xanthan gum at concentrations of up to 1500 mg/L (232 mg/kg bw per day) were well tolerated.

A NOAEL of 750 mg/kg bw per day (provided as a formulation of 1500 mg/L) was established for xanthan gum in neonatal pigs. The Committee considers that the neonatal pig is an appropriate animal model for the assessment of the safety of the additive for infants; the neonatal pigs are fed the xanthan gum–containing test formulations during the first 3 weeks of life (starting 2 days after birth) as the sole source of nutrition to model the 0- to 12-week period of development in human infants in which infant formula may be provided as the sole source of nutrition.

The MOE based on this NOAEL and the conservative estimate of xanthan gum intake of 220 mg/kg bw per day by infants (high energy requirements for fully formula-fed infants) is 3.4. The Committee previously commented that when relevant uncertainties or conservatisms in the toxicological data and/or the exposure estimates were taken into account, an MOE in the region of 1–10 could be interpreted as indicating low risk for the health of 0- to 12-week-old infants consuming the food additive in infant formula (Annex 1, reference *220*). The relevant considerations in relation to the evaluation of xanthan gum for use in infant formula are as follows:

- The toxicity of xanthan gum is low.
- The NOAEL is derived from two studies in neonatal pigs, which are considered a relevant animal model for human infants.
- Clinical studies in infants support the tolerability of formula containing concentrations of xanthan gum up to 1500 mg/L.
- No adverse events were reported in post-marketing surveillance conducted by one manufacturer over a 5-year period on formulas containing xanthan gum at concentrations up to 1000 mg/L.

Based on these considerations, the Committee concluded that the consumption of xanthan gum in infant formula or formula for special medical purposes intended for infants is of no safety concern at the maximum proposed use level of 1000 mg/L, leading to the conservative estimate of dietary exposure of 220 mg/kg bw per day. The Committee recognizes that there is variability in medical conditions among infants requiring formula for special medical purposes and that these infants would normally be under medical supervision.

A consolidated monograph was prepared.

At the present meeting, the Committee reviewed the specifications for xanthan gum. 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 2.3.3. The Committee maintained the limit for lead in xanthan gum at 2 mg/kg for general use and introduced a limit for lead of 0.5 mg/kg for use in infant formula.

The Committee also noted that the test method for the determination of residual solvents currently employs a gas chromatographic method using a packed column. The Committee replaced this method with a method using a capillary column.

The specifications were revised, and a Chemical and Technical Assessment was prepared.

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3.2 Revision of specifications

3.2.1 Aspartame

At the present meeting, the Committee considered all information necessary for revision of the specifications for aspartame, in particular change of test for 5-benzyl-3,6-dioxo-2-piperazineacetic acid and change of test for other optical isomers.

The purity tests for 5-benzyl-3,6-dioxo-2-piperazineacetic acid and other optical isomers were replaced by new published and validated HPLC tests.

The Committee received information relating to specifications for the solubility of aspartame in ethanol. As a result, the identification characteristic for solubility in ethanol was changed from "slightly soluble" to "practically insoluble or insoluble".

3.2.2 Cassia gum

The Secretariat received a comment that the method of analysis for anthraquinones provided in the specifications for cassia gum prepared at the seventy-third meeting of the Committee (Annex 1, reference *202*) and published in FAO JECFA Monographs 10 (Annex 1, reference *204*) was not suitable for the accurate analysis of this group of compounds. The commenter further stated that a suitable method may be found in the submission FAD-2009-0056 [EFSA/JRC/IMRR/EURL (former CRL)/European Commission according to Regulation

(EC) 1831/2003] for the analysis of anthraquinones in cassia gum preparations used in animal feed.

A request for information on a suitable method of analysis for anthraquinones in cassia gum was added to the call for data for the present meeting. No information was received in response to this call. In addition, the original sponsor of the cassia gum specifications was no longer able to comment on the suitability of the methodology, as the company no longer manufactures the product. The Committee decided to remove the current method for anthraquinones from the specifications and make the specifications tentative.

The Committee noted that the substance can be obtained from a number of companies (according to Internet searches) and requested information on validated methods of analysis currently in use by providers of cassia gum. The methods submitted should contain details of the use of standard (reference) materials, the extraction efficiency of the initial steps, the recovery of the analytes in question, performance data and the results of the analysis of several batches of the material in commerce.

The tentative specifications will be withdrawn unless the requested information is submitted before 31 December 2017.

3.2.3 Citric and fatty acid esters of glycerol (CITREM)

At the request of CCFA at its Forty-seventh Session *(9)*, citric and fatty acid esters of glycerol (CITREM) (INS No. 472c) was on the agenda at the present meeting to assess data on the levels of lead and to consider a specific limit for the lead specifications when the additive is intended for use as an emulsifier in infant formula and formula in the category of foods for special medical purposes intended for infants (infant formulas). The Committee reviewed data relevant to the use of CITREM in infant formulas.

The Committee discussed the data provided for lead levels in the manufacture of CITREM, as described in section 2.3.3. The Committee revised the CITREM specifications by introducing a limit for lead of 0.5 mg/kg for use in infant formula.

The Committee at its seventy-ninth meeting (Annex 1, reference *220*) had recommended replacing the packed column gas chromatography test method for the determination of total citric acid. The present Committee recommends that data be submitted for the replacement of this method with a suitable method using a capillary/wide-bore column for consideration at a future meeting, as described in section 2.3.1.

3.2.4 Modified starches

The Committee at the seventy-ninth meeting (Annex 1, reference *220*) recommended the separation of the combined specification for the modified starches into 16 separate specifications. As a first step, the modifications were separated into 16 stand-alone documents without adding, deleting or modifying any information. Some of the resulting individual draft specifications monographs were incomplete; in some cases, essential information was missing, in particular information that would normally be needed to unambiguously characterize the additive. Therefore, a revision of the individual draft specifications monographs was required. The data and information necessary to complete and revise the 16 individual draft specifications monographs were requested through a call for data.

Based on the limited information received, the Committee was able to prepare full specifications for the following three modified starches:

1) oxidized starch (INS No. 1404),
2) starch acetate (INS No. 1420) and
3) acetylated oxidized starch (INS No. 1451).

The Committee prepared tentative specifications for the following 13 modified starches and requires the following information for the removal of the tentative status:

Modified starch	Information required on
Dextrin roasted starch (INS No. 1400)	• A suitable method for the Dispersion or Reducing Sugars Distinguishing Test
Acid treated starch (INS No. 1401)	• A suitable method for the Dispersion or Reducing Sugars Distinguishing Test
Alkaline treated starch (INS No. 1402)	• A suitable method for the Dispersion or Reducing Sugars Distinguishing Test
Bleached starch (INS No. 1403)	• Typical levels of residual reagents or by-products
Enzyme-treated starch (INS No. 1405)	• A suitable method for the Dispersion or Reducing Sugars Distinguishing Test
Monostarch phosphate (INS No. 1410)	• A suitable test for identification of the phosphate groups
Distarch phosphate (INS No. 1412)	• A suitable test for identification of the phosphate groups and of crosslinking
Phosphated distarch phosphate (INS No. 1413)	• A suitable test for identification of the phosphate groups and of crosslinking
Acetylated distarch phosphate (INS No. 1414)	• A suitable test for identification of the phosphate groups and of crosslinking
Acetylated distarch adipate (INS No. 1422)	• A suitable test for identification of the adipate groups • Levels of free adipic acid
Hydroxypropyl starch (INS No. 1440)	• A suitable method for the determination of propylene chlorohydrin
Hydroxypropyl distarch phosphate (INS No. 1442)	• A suitable method for the determination of propylene chlorohydrin • A suitable test for identification of the phosphate groups
Starch sodium octenyl succinate (INS No. 1450)	• A suitable test for identification of octenylsuccinate groups

The Committee noted that all the modified starches may additionally be subjected to bleaching and therefore included the appropriate purity tests in the revised specifications.

The Committee recommended that the call for data also include method of manufacture for each of the 16 modified starches. The missing data are required by 31 December 2017.

Starch sodium octenyl succinate (INS. No. 1450) was also listed in the call for data for the eighty-second meeting, requesting data on lead levels for use in infant formula (see sections 2.3.3 and 3.2.6).

3.2.5 Octanoic acid

The Committee at its sixty-third meeting (Annex 1, reference *173*) evaluated octanoic acid as a component of antimicrobial washing solutions. Octanoic acid was on the agenda at the present meeting for the incorporation of the infrared spectrum identity test into the specifications monograph.

The specifications for octanoic acid were revised to include infrared test conditions and the reference spectrum.

3.2.6 Starch sodium octenyl succinate

Starch sodium octenyl succinate (INS No. 1450) was on the agenda of the current meeting at the request of CCFA at its Forty-seventh Session *(9)* to assess the data on the levels of lead when the additive is intended for use in infant formula and formula in the category of food for special medical purposes intended for infants and to consider a specific limit in the specifications. The Committee did not receive any data in response to the call for data. The limit for lead (2 mg/kg) in the specifications was maintained.

The Committee also discussed the limits of lead in specifications for additives, at proposed use levels, for use in infant formula, as described in section 2.3.3.

Starch sodium octenyl succinate (INS No. 1450) was also on the agenda with respect to the revision of specifications for the modified starches (refer to section 3.2.4 of the report).

3.2.7 Total colouring matters

At the present meeting, total colouring matters content (tentative) (Vol. 4) was revised by amending Procedure 1 (water-soluble colouring matters) and Procedure 3 (lakes). Table 1 was revised to give spectrophotometric data for 17 synthetic colours, their aluminium lakes, cochineal extract and carmine dissolved in water and buffers. Reagents, solution preparations and sample preparation

information were added. Equations shown in Procedures 1, 2 and 3 were edited. The tentative status of the method was removed.

In addition, where available, information on the wavelength of maximum absorbance, absorptivity and/or specific absorbance (including information on the solvent used) for the 17 synthetic colours and cochineal extract used to form a lake was included in Table 1 of the revised method. The objective was to provide single values for each synthetic colour to allow for the establishment of consensus values.

Although data were not requested for Procedure 2 (organic solvent–soluble colouring matters), the Committee noted that chloroform is listed as a reagent in that procedure. The Committee was reminded of previous efforts to remove this reagent from test procedures and decided that efforts should be made to replace it (see also section 2.3.4).

4. Flavouring agents

4.1 Flavouring agents evaluated by the Procedure for the Safety Evaluation of Flavouring Agents[4,5]

Assignment to structural class

Five groups of flavouring agents were evaluated using the Procedure for the Safety Evaluation of Flavouring Agents, as outlined in Fig. 2 (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.

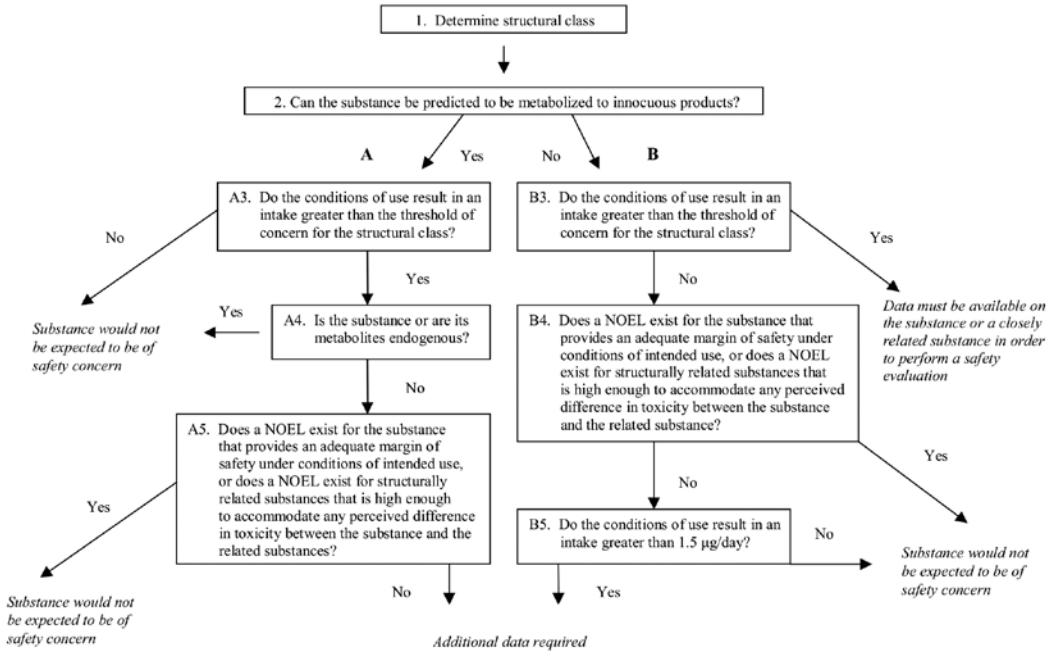
A key element of the Procedure involves determining whether a flavouring agent and the product(s) of its metabolism are innocuous and/or endogenous substances. For the purpose of the evaluations, the Committee used the following definitions, adapted from the report of its forty-sixth meeting (Annex 1,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

[4] The Committee revised the Procedure for the Safety Evaluation of Flavouring Agents at the current meeting (see section 2.2.1) and concluded that the revised Procedure for the Safety Evaluation of Flavouring Agents should be applied in its future evaluations.

[5] Numbered references cited in the subsections of section 4.1 are provided at the end of each subsection.

Fig. 2
Procedure for the Safety Evaluation of Flavouring Agents



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

Assessment of dietary exposure

Maximized survey-derived intake (MSDI)

Estimates of the dietary exposure to flavouring agents by populations are based on annual volumes of production. These data were derived from surveys in Europe, Japan and the 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.[6]

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:

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

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/.

[6] Population counts in 2010 were reported by the International Organization of the Flavor Industry *(10)* to be 410 million for Europe (EU-16 plus Turkey and Switzerland), 309 million for the USA and 128 million for Japan.

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 Alicyclic, alicyclic-fused and aromatic-fused ring lactones

Introduction

The Committee evaluated an additional two flavouring agents belonging to the group of alicyclic, alicyclic-fused and aromatic-fused ring lactones that was evaluated previously. The additional flavouring agents are a gamma-lactone fused to an alicyclic ring (No. 2223) and a delta-lactone fused to a benzene ring (No. 2224). The evaluations were conducted using the Procedure for the Safety Evaluation of Flavouring Agents (Annex 1, reference *131*). These two flavouring agents have not previously been evaluated by the Committee.

The Committee previously evaluated 16 other members of this group of flavouring agents at its sixty-first meeting (Annex 1, reference *166*). The Committee concluded that all 16 flavouring agents in that group were of no safety concern at estimated dietary exposures.

One of the additional flavouring agents in this group, No. 2223, has been reported to occur as a natural component of orange and grapefruit juice and fresh apples [1].

Assessment of dietary exposure

The total annual volumes of production of the two flavouring agents belonging to the group of alicyclic, alicyclic-fused and aromatic-fused ring lactones are approximately 0.1 kg in Europe, 0.9 kg in the USA and 0.1 kg in Japan [2]. Greater than 99% of the total annual volume in Europe and Japan and approximately 90% of the total annual volume in the USA are accounted for by 2-(2-hydroxy-4-methyl-3-cyclohexenyl)propionic acid gamma-lactone (No. 2223).

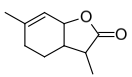
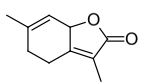
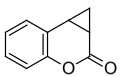
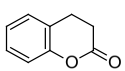
Dietary exposures were estimated using the MSDI method and the SPET. The highest estimated dietary exposure for each flavouring agent is reported in Table 1. The estimated daily dietary exposure is highest for 2-(2-hydroxy-4-methyl-3-cyclohexenyl)propionic acid gamma-lactone (No. 2223) (300 µg/day, the SPET value obtained for instant coffee and tea) [3]. For the other flavouring agent, the SPET also yielded the highest estimate.

Absorption, distribution, metabolism and elimination

Information on the absorption, distribution, metabolism and elimination of flavouring agents belonging to the group of alicyclic, alicyclic-fused and aromatic-

Table 1

Summary of the results of the safety evaluations of alicyclic, alicyclic-fused and aromatic-fused ring lactones 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								
2-(2-Hydroxy-4-methyl-3-cyclohexenyl)propionic acid gamma-lactone	2223	57743-63-2 	Yes, SPET: 300	No	Yes. The NOAEL of 1 mg/kg bw per day for the structurally related dehydromenthofurolactone in a 90-day study in rats [5] is 200 times the estimated dietary exposure to No. 2223 when used as a flavouring agent.	Note 1	Dehydromentho-furolactone (No. 1163) 	No safety concern
2-(2-Hydroxyphenyl)-cyclopropanecarboxylic acid delta-lactone	2224	5617-64-1 	Yes, SPET: 250	No	Yes. The NOAEL of 150 mg/kg bw per day for the structurally related dihydrocoumarin in a 90-day study in rats [6] is 38 000 times the estimated dietary exposure to No. 2224 when used as a flavouring agent.	Note 2	Dihydrocoumarin (No. 1171) 	No safety concern

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

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

[c] *Step 2*: Both 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 values calculated by either the SPET or MSDI method. The SPET gave the highest estimated dietary exposure in each case.

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

Notes:

1. Hydrolysed to the open-chain hydroxycarboxylic acid derivative and excreted, or oxidative degradation of the carboxylic acid side-chain to yield polar alicyclic or aromatic carboxylic acids that are excreted unchanged or in conjugated form. Ring alkyl substituents may be hydroxylated, producing a more polar metabolite, which is then excreted.
2. Aromatic fused-ring lactones hydrolysed to the open-chain hydroxycarboxylic acid derivative and excreted as glycine and/or glutamine conjugates. In addition, oxidation or reduction of the side-chain and subsequent excretion as the glucuronic acid conjugate.

fused ring lactones has previously been described in the report of the sixty-first meeting (Annex 1, reference *166*). 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, the Committee assigned both flavouring agents (Nos 2223 and 2224) to structural class III [4].

Step 2. The two 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 for the two flavouring agents are above the threshold of concern (i.e. 90 µg/day for class III). Accordingly, the evaluation of these flavouring agents proceeded to step A4.

Step A4. The two flavouring agents and their metabolites are not endogenous, and therefore the evaluations proceeded to step A5.

Step A5. For 2-(2-hydroxy-4-methyl-3-cyclohexenyl)propionic acid gamma-lactone (No. 2223), the NOAEL[7] of 1 mg/kg bw per day for the structurally related dehydromenthofurolactone (No. 1163) obtained from a 90-day study in rats [5] (Annex 1, reference *167*) provides an adequate MOE of 200 in relation to the highest estimated dietary exposure to No. 2223 (SPET = 300 µg/day or 5 µg/kg bw per day) when used as a flavouring agent. The Committee therefore concluded that 2-(2-hydroxy-4-methyl-3-cyclohexenyl)propionic acid gamma-lactone (No. 2223) would not pose a safety concern at current estimated dietary exposures.

For 2-(2-hydroxyphenyl)cyclopropanecarboxylic acid delta-lactone (No. 2224), the NOAEL[7] of 150 mg/kg bw per day for the structurally related dihydrocoumarin (No. 1171) obtained from a 90-day study in rats [6] (Annex 1, reference *167*) provides an adequate MOE of 38 000 in relation to the estimated dietary exposure to No. 2224 (SPET = 250 µg/day or 4 µg/kg bw per day) when used as a flavouring agent. The Committee therefore concluded that 2-(2-hydroxyphenyl)cyclopropanecarboxylic acid delta-lactone (No. 2224) would not pose a safety concern at current estimated dietary exposures.

Table 1 summarizes the evaluations of the two flavouring agents belonging to this group of alicyclic, alicyclic-fused and aromatic-fused ring lactones (Nos 2223 and 2224).

[7] Prior to the sixty-eighth meeting of the Committee (Annex 1, reference 187), this NOAEL would have been termed a NOEL.

Consideration of combined intakes from use as flavouring agents

The two additional flavouring agents in this group of alicyclic, alicyclic-fused and aromatic-fused ring lactones have low MSDI values (0.01–0.09 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.

Consideration of secondary components

One flavouring agent in this group (No. 2224) has a minimum assay value of less than 95% (see Annex 3). The major secondary component, dihydrocoumarin (No. 1171), present at 2–3%, is considered not to present a safety concern at estimated dietary exposures from use of No. 2224 as a flavouring agent.

Conclusions

In the previous evaluation of flavouring agents in this group of alicyclic, alicyclic-fused and aromatic-fused ring lactones, studies of hydrolysis, absorption, distribution, metabolism, elimination, acute toxicity, short-term and long-term toxicity, and genotoxicity were available. None of the 16 previously evaluated flavouring agents raised safety concerns.

For a previously evaluated flavouring agent in this group (No. 1166), a study of acute toxicity and two studies of genotoxicity were available. The additional data raised no safety concerns and supported the previous evaluation.

For the present evaluation of two additional flavouring agents belonging to this group (Nos 2223 and 2224), studies of acute toxicity and genotoxicity were available for No. 2223. Studies of short-term toxicity on previously evaluated flavouring agents that are structurally related to Nos 2223 and 2224 supported the safety evaluation of these flavouring agents.

The Committee concluded that the two flavouring agents (Nos 2223 and 2224) that are additions to the group of alicyclic, alicyclic-fused and aromatic-fused ring lactones evaluated previously would not give rise to safety concerns at current estimated dietary exposures.

An addendum to the monograph was prepared.

References

1. Nijssen LM, Ingen-Visscher CAV, Donders JJH. Volatile compounds in food 16.1 [database]. Zeist: TNO Triskelion; 2015 (http://www.vcf-online.nl/VcfHome.cfm).

2. 2010 IOFI global poundage survey. Geneva: International Organization of the Flavor Industry; 2013. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.

3. International Organization of the Flavor Industry. Interim inquiry on volume use and added use levels for flavoring agents to be presented at the JECFA 82nd meeting. Private communication to the

Flavor and Extract Manufacturers Association, Washington, DC, USA; 2015. Submitted to WHO by the International Organization of the Flavor Industry, Brussels, Belgium.

4. Cramer GM, Ford RA, Hall RL. Estimation of toxic hazard – a decision tree approach. Food Cosmet Toxicol. 1978;16:255–76.

5. Voss KA. 90-day dietary toxicity study of SRA 84-11 in Sprague-Dawley rats (dehydromenthofurolactone). Unpublished report to the Flavor and Extract Manufacturers Association; 1985. Submitted to WHO by the International Organization for the Flavor Industry, Brussels, Belgium.

6. Toxicology and carcinogenesis studies of 3,4-dihydrocoumarin (CAS 119-84-6) in F344/N rats and B6C3F1 mice (gavage studies). Research Triangle Park (NC): United States Department of Health and Human Services, Public Health Service, National Institutes of Health, National Toxicology Program; 1993 (NTP Technical Report Series No. 423).

4.1.2 Aliphatic and aromatic amines and amides

Introduction

The Committee evaluated nine flavouring agents (five new additions and four re-evaluations) belonging to the group of aliphatic and aromatic amines and amides. The five additional flavouring agents include one oxalamide (No. 2225), one benzamide (No. 2226), two propenamides (Nos 2227 and 2228) and one menthyl carboxamide (No. 2229). All of the evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents (Annex 1, reference *131*). None of these new flavouring agents has previously been evaluated by the Committee. Four of the five new flavouring agents in this group (Nos 2225–2228) are reported to be flavour modifiers.

The four flavouring agents presented for re-evaluation (Nos 1595, 2005, 2010 and 2011) are branched-chain alkyl carboxamides. No. 1595 was previously evaluated by the Committee together with 36 other members of this group of flavouring agents at its sixty-fifth meeting (Annex 1, reference *178*). For one of these flavouring agents (No. 1592), the Committee considered it inappropriate for use as a flavouring agent or for food additive purposes based on available data indicating carcinogenicity in mice and rats. For the remaining 36 flavouring agents, including No. 1595, the Committee concluded that they would not give rise to safety concerns based on estimated dietary exposures. However, as the dietary exposure estimates for 27 of these flavouring agents were based on anticipated annual volumes of production, these evaluations were conditional pending submission of use levels or poundage data, which were provided at the sixty-ninth meeting (Annex 1, reference *190*). For No. 1595, additional data available at the sixty-ninth meeting raised safety concerns, and the Committee concluded that the Procedure could not be applied to this flavouring agent until additional safety data became available. Data requested included data on the potential of this compound to form reactive metabolites and on whether clastogenicity is also

expressed in vivo, as well as additional information on the kidney effects found at relatively low doses (Annex 1, reference *190*).

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

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

The Committee evaluated another seven members of this group of flavouring agents at its seventy-sixth meeting and concluded that all seven were of no safety concern at estimated dietary exposures (Annex 1, reference *211*).

At the current meeting, additional safety data on No. 1595 were submitted, and it was proposed that No. 1595 be used as a structurally related substance in support of the safety evaluation of flavouring agents Nos 2005, 2010 and 2011.

None of the nine flavouring agents considered at the current meeting has been reported to occur naturally in foods.

Assessment of dietary exposure

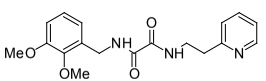
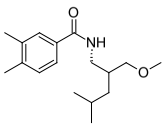
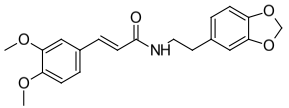
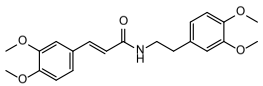
The total annual volume of production of the five new flavouring agents belonging to the group of aliphatic and aromatic amines and amides is approximately 0.7 kg in the USA [1].

The total annual volume of production of the four flavouring agents presented for re-evaluation is approximately 4 kg in Europe and 83 186 kg in the USA [2]. The entire volume (100%) of the annual production in Europe and more than 99% of the annual production volume in the USA are accounted for by one flavouring agent, No. 1595.

Dietary exposures were estimated using both the MSDI method and the SPET, with the highest values reported in Table 2. The estimated daily dietary exposure is highest for No. 2010 (48 000 μg/day, SPET value). For the other flavouring agents, daily dietary exposures ranged from 0.01 to 27 000 μg/day, with the SPET yielding the highest estimate in all but one case (No. 1595).

Table 2

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

Flavouring agent	No.	CAS No. and structure	*Step 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	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
Structural class III							
N1-(2,3-Dimethoxybenzyl)-*N2*-(2-(pyridin-2-yl)ethyl)-oxalamide	2225	851670-40-18 	Yes, SPET: 2 000	Yes. No. 2225 is non-genotoxic in bacteria, and the NOAEL of 140 mg/kg bw per day (the highest dose tested) in a 28-day study in rats [12] is 4 200 times the estimated dietary exposure to No. 2225 when used as a flavouring agent.	Note 1	–	No safety concern
(*R*)-*N*-(1-Methoxy-4-methylpentan-2-yl)-3,4-dimethylbenzamide	2226	851669-60-8 	Yes, SPET: 800	Yes. No. 2226 is non-genotoxic in bacteria and in mammalian cells in vitro and in vivo, and the NOAEL of 100 mg/kg bw per day (the highest dose tested) in a 28-day study in rats [16] is 7 700 times the estimated dietary exposure to No. 2226 when used as a flavouring agent.	Note 2	–	No safety concern
(*E*)-*N*-[2-(1,3-Benzodioxol-5-yl)ethyl]-3-(3,4-dimethoxyphenylprop-2-enamide	2227	125187-30-6 	Yes, SPET: 400	Yes. No. 2227 and the structurally related *N*-[2-(3,4-dimethoxyphenyl)-ethyl]-3,4-dimethoxycinnamic acid amide (No. 1777) are non-genotoxic in bacteria, and the NOAEL of 69 mg/kg bw per day for No. 1777 in a 90-day study in rats [18] is 9 900 times the estimated dietary exposure to No. 2227 when used as a flavouring agent.	Note 1	*N*-[2-(3,4-Dimethoxyphenyl)ethyl]-3,4-dimethoxycinnamic acid amide (No. 1777) 	No safety concern

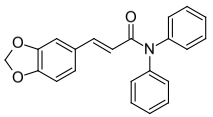
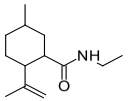
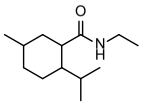
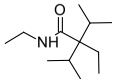
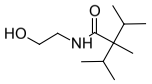
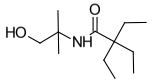
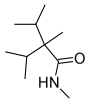
Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does estimated dietary exposure exceed the threshold of concern?	*Follow-on from step B3*[e] AAre additional data available for the flavouring agent with an estimated dietary exposure exceeding the threshold of concern?	Comments on predicted metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
(*E*)-3-Benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide	2228	1309389-73-8 	Yes, SPET: 100	Yes. No. 2228 is non-genotoxic in bacteria, and the NOAEL of 490 mg/kg bw per day (the highest dose tested) in a 90-day study in rats [20] is 245 000 times the estimated dietary exposure to No. 2228 when used as a flavouring agent.	Note 1	–	No safety concern
N-Ethyl-5-methyl-2-(methylethenyl)-cyclohexanecarboxamide	2229	1582789-90-9 	Yes, SPET: 15 000	Yes, for the structurally related *N*-ethyl 2-isopropyl-5-methylcyclohexane-carboxamide (No. 1601). This compound is of low acute toxicity, and from four short-term toxicity studies available, the overall NOAEL of 8 mg/kg bw per day in a 28-day study in rats [21] is 32 times the SPET estimate and 40 million times the MSDI when No. 2229 is used as a flavouring agent.	Note 1	*N*-Ethyl 2-isopropyl-5-methyl-cyclohexanecarboxamide (No. 1601) 	Additional data required to complete evaluation
N-Ethyl-2,2-diisopropylbutanamide	2005	51115-70-9 	Yes, SPET: 27 000	No	Note 1	–	Additional data required to complete evaluation
N-(2-Hydroxyethyl)-2,3-dimethyl-2-isopropylbutanamide	2010	883215-02-9 	Yes, SPET: 48 000	No	Notes 1 and 3	–	Additional data required to complete evaluation

Table 2 (continued)

Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does estimated dietary exposure exceed the threshold of concern?	*Follow-on from step B3*[e] AAre additional data available for the flavouring agent with an estimated dietary exposure exceeding the threshold of concern?	Comments on predicted metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
N-(1,1-Dimethyl-2-hydroxyethyl)-2,2-diethylbutanamide	2011	51115-77-6 	Yes, SPET: 27 000	No	Notes 1 and 3	–	Additional data required to complete evaluation
Flavouring agent not evaluated according to the Procedure							
2-Isopropyl-*N*,2,3-trimethylbutyramide	1595	51115-67-4 	Concerns for in vivo genotoxicity and kidney effects at low doses				

[a] Sixty-five flavouring agents in this group were previously evaluated by the Committee (Annex 1, references *178*, *187*,*190*, *202* and *211*).

[b] *Step 1*: The five additional flavouring agents in this group (Nos 2225–2229) are in structural class III, as are the three flavouring agents in this group presented for re-evaluation.

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

[d] The threshold for human dietary exposure for structural class III is 90 µg/day. All dietary exposure values are expressed in µg/day. The dietary exposure 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. In cases where the resulting MOE was relatively low, a comparison with the MSDI was also made.

Notes:

1. Amides are expected to undergo limited hydrolysis and/or oxidation and enter into known pathways of metabolism and excretion.
2. Extensive metabolism of No. 2226 was observed in vivo, involving hydroxylation, dihydroxylation, demethylation and glucuronidation.
3. It is anticipated that the free hydroxyl group will form conjugates with sulfate or glucuronic acid, followed by excretion in the urine.

Absorption, distribution, metabolism and elimination

Information on the absorption, distribution, metabolism and elimination of flavouring agents belonging to the group of aliphatic and aromatic amines and amides has previously been described in the monographs of the sixty-fifth, sixty-eighth, seventy-third and seventy-sixth meetings (Annex 1, references *179*, *188*, *203* and *212*).

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

In relation to the additional flavouring agents considered at the current meeting of the Committee, only limited information regarding metabolic pathways is available for specific substances. Pharmacokinetic studies on *N1*-(2,3-dimethoxybenzyl)-*N2*-(2-(pyridin-2-yl)ethyl)oxalamide (No. 2225) and (*R*)-*N*-(1-methoxy-4-methylpentan-2-yl)-3,4-dimethylbenzamide (No. 2226) indicated rapid elimination from plasma following oral administration to rats, although bioavailability was poor for (*R*)-*N*-(1-methoxy-4-methylpentan-2-yl)-3,4-dimethylbenzamide [3, 4]. Metabolic biotransformation of (*R*)-*N*-(1-methoxy-4-methylpentan-2-yl)-3,4-dimethylbenzamide involved hydroxylation, dihydroxylation, demethylation and glucuronidation [5].

Flavouring agent not evaluated according to the Procedure for the Safety Evaluation of Flavouring Agents at current meeting

For 2-isopropyl-*N*,2,3-trimethylbutyramide (No. 1595), the toxicity data available to the Committee when re-evaluating this flavouring agent at its sixty-ninth meeting (two acute studies, a 14-day toxicity study, three 90-day/14-week toxicity studies, and a study of reproductive toxicity and teratogenicity [all in rats], as well as several studies of genotoxicity in vitro) raised safety concerns, and it was concluded that the Procedure could not be applied to this flavouring agent until additional safety data became available. To address the concern for possible in vivo genotoxicity of No. 1595, the Committee at its sixty-ninth meeting requested data on the potential of this compound to form reactive metabolites and on whether clastogenicity is also expressed in vivo. In response to this request, three in vivo studies of genotoxicity were provided to the Committee at its present meeting. In these studies, No. 1595 did not induce chromosome aberrations in rat bone marrow cells [6] or comet effects in female rat kidney cells [7], whereas it was weakly genotoxic in the comet assay in male rat kidney cells [8]. It was postulated that this effect was male specific, given that histopathology in the same study

revealed an increase in the severity of hyaline droplets in the tubular epithelium of kidneys. The Committee, however, noted the absence of both histopathological examination of the kidneys in the female comet assay and other data informing on the difference in results between male and female rats. The Committee further noted that no data were provided on the potential of this compound to form reactive metabolites. Additional information on the (inconsistent) kidney effects observed in the studies of short-term toxicity at relatively low doses, in response to the second request of the Committee at its sixty-ninth meeting, was also not received.

Hence, the present Committee concluded that the concerns previously expressed by the Committee at its sixty-ninth meeting as to in vivo genotoxicity and how to address the kidney effects and identify a NOAEL have not been sufficiently addressed and that the Procedure still could not be applied to No. 1595.

Information that would assist in resolving the concerns would include data informing on the difference in response observed in the kidney of male and female rats in the comet assay and on the potential of this compound to form reactive metabolites, as well as additional information on the kidney effects found at relatively low doses.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

The evaluations for Nos 2225–2229, 2005, 2010 and 2011 were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents, as described below.

Step 1. In applying the Procedure to the above-mentioned flavouring agents, the Committee assigned all five additional flavouring agents (Nos 2225–2229) and the three flavouring agents for re-evaluation (Nos 2005, 2010 and 2011) to structural class III [9].

Step 2. All eight flavouring agents (Nos 2225–2229, 2005, 2010 and 2011) in this group cannot be predicted to be metabolized to innocuous products. Therefore, the evaluation of these flavouring agents proceeded via the B-side of the Procedure.

Step B3. The highest estimated dietary exposures for all eight flavouring agents in this group are above the threshold of concern (i.e. 90 μg/day for class III). Accordingly, data must be available on these flavouring agents or closely related substances 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 Nos 2225–2229, 2005, 2010 and 2011, as their estimated dietary exposures exceeded the threshold of concern for structural class III (90 μg/day).

For *N1*-(2,3-dimethoxybenzyl)-*N2*-(2-(pyridin-2-yl)ethyl)oxalamide (No. 2225), pharmacokinetic data, a genotoxicity study and a 28-day toxicity study were available. This flavouring agent was found to be rapidly absorbed and eliminated from plasma, with moderate oral bioavailability [3]. The flavouring agent was negative for bacterial mutagenicity with and without an exogenous activation system [10, 11]. The NOAEL of 140 mg/kg bw per day (the highest dose tested) in a 28-day toxicity study in rats [12] provides an adequate MOE of 4200 in relation to the estimated dietary exposure to No. 2225 (SPET = 2000 μg/day or 33 μg/kg bw per day) when used as a flavouring agent. The Committee concluded that, on the basis of all of the available evidence, No. 2225 would not pose a safety concern at current estimated dietary exposures.

For (*R*)-*N*-(1-methoxy-4-methylpentan-2-yl)-3,4-dimethylbenzamide (No. 2226), pharmacokinetic data, a 28-day toxicity study and genotoxicity studies were available. This flavouring agent was found to be poorly bioavailable and rapidly eliminated from plasma [4]. The flavouring agent was negative for bacterial mutagenicity with and without an exogenous activation system [13], for chromosome aberrations in human peripheral blood lymphocytes with and without an exogenous activation system [14] and for induction of micronuclei in mouse bone marrow erythrocytes [15]. The NOAEL of 100 mg/kg bw per day (the highest dose tested) in a 28-day toxicity study in rats [16] provides an adequate MOE of approximately 7700 in relation to the estimated dietary exposure to No. 2226 (SPET = 800 μg/day or 13 μg/kg bw per day) when used as a flavouring agent. The Committee concluded that, on the basis of all of the available evidence, No. 2226 would not pose a safety concern at current estimated dietary exposures.

For (*E*)-*N*-[2-(1,3-benzodioxol-5-yl)ethyl]-3-(3,4-dimethoxyphenyl)-prop-2-enamide (No. 2227), only a genotoxicity study was available. The flavouring agent was negative for bacterial mutagenicity with and without an exogenous activation system [17]. The NOAEL of 69 mg/kg bw per day for the structurally related *N*-[2-(3,4-dimethoxyphenyl)ethyl]-3,4-dimethoxycinnamic acid amide (No. 1777) in a 90-day study in rats [18] provides an adequate MOE of approximately 9900 in relation to the estimated dietary exposure to No. 2227 (SPET = 400 μg/day or 7 μg/kg bw per day) when used as a flavouring agent. The Committee concluded that, on the basis of all of the available evidence, No. 2227 would not pose a safety concern at current estimated dietary exposures.

For (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide (No. 2228), a genotoxicity study and a 90-day toxicity study were available. The flavouring agent was negative for bacterial mutagenicity with and without an exogenous activation system [19]. The NOAEL of 490 mg/kg bw per day (the highest dose tested) in a 90-day toxicity study in rats [20] provides an adequate MOE of 245 000 in relation to the estimated dietary exposure to No. 2228 (SPET

= 100 µg/day or 2 µg/kg bw per day) when used as a flavouring agent. The Committee concluded that, on the basis of all of the available evidence, No. 2228 would not pose a safety concern at current estimated dietary exposures.

For *N*-ethyl-5-methyl-2-(methylethenyl)cyclohexanecarboxamide (No. 2229), no substance-specific data are available. The NOAEL of 8 mg/kg bw per day for the structurally related *N*-ethyl 2-isopropyl-5-methylcyclohexanecarboxamide (No. 1601) in a 28-day study in rats [21] is 32 times the SPET estimate (15 000 µg/day or 250 µg/kg bw per day) and 40 million times the MSDI (0.01 µg/day or 0.0002 µg/kg bw per day) when No. 2229 is used as a flavouring agent. The Committee therefore concluded that the NOAEL does not provide an adequate MOE based on the SPET and that additional data are required to complete the evaluation.

For *N*-ethyl-2,2-diisopropylbutanamide (No. 2005), *N*-(2-hydroxyethyl)-2,3-dimethyl-2-isopropylbutanamide (No. 2010) and *N*-(1,1-dimethyl-2-hydroxyethyl)-2,2-diethylbutanamide (No. 2011), NOAELs for these flavouring agents or structurally related substances were not available. Although 2-isopropyl-*N*,2,3-trimethylbutyramide (No. 1595) is structurally related, the present Committee concluded that No. 1595 could not be evaluated using the Procedure, and therefore this flavouring agent was not suitable to support the evaluation of these three flavouring agents. Therefore, for these three flavouring agents, the Committee concluded that additional data would be necessary to complete the evaluation.

Table 2 summarizes the evaluations of the additional five flavouring agents (Nos 2225–2229) and the re-evaluations of the three flavouring agents previously evaluated (Nos 2005, 2010 and 2011) in this group of aliphatic and aromatic amines and amides.

Consideration of combined intakes from use as flavouring agents

The five additional flavouring agents in the group of aliphatic and aromatic amines and amides have low MSDIs (0.01–0.03 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 additional data on previously evaluated flavouring agents

For some of the previously evaluated flavouring agents in this group, additional studies of short-term toxicity (Nos 1598, 1600, 1776, 1777, 2006 and 2077) and genotoxicity (Nos 1776 and 2009) were available for this meeting. The additional studies in general support the previous safety evaluations for these flavouring agents. The Committee noted, however, that for two of the previously evaluated

flavouring agents (Nos 1598 and 2077) evaluated by the B-side of the Procedure, the NOAELs identified in the newly provided short-term toxicity studies in rats (10 and 23 mg/kg bw per day, respectively) were lower than the NOAELs used by the Committee for their previous safety evaluations at the sixty-fifth and sixty-ninth (No. 1598) and seventy-sixth (No. 2077) meetings (572 mg/kg bw per day for both). Compared with their dietary exposures as estimated at this meeting, the new NOAELs provide MOEs of 200 for No. 1598 (SPET = 3000 μg/day or 50 μg/kg bw per day) and approximately 300 for No. 2077 (SPET = 4500 μg/day or 75 μg/kg bw per day).

Conclusion

In the previous evaluations of members of this group of flavouring agents, studies of acute toxicity, short-term studies of toxicity, long-term studies of toxicity and carcinogenicity, and studies of genotoxicity and reproductive toxicity were available. For some previously evaluated flavouring agents in this group, additional toxicity data were available for this meeting. These additional data were generally in support of the previous safety evaluations. For Nos 1598 and 2077, the new studies resulted in lower NOAELs. In light of general considerations on the Procedure for the Safety Evaluation of Flavouring Agents and the need for an approach for re-evaluation in light of new data (see sections 2.2.1 and 2.2.2), the Committee recommends re-evaluation of these two flavouring agents at a future meeting.

For the present evaluation of five flavouring agents that are additions to this group (Nos 2225–2229), data from studies of short-term toxicity (Nos 2225, 2226 and 2228) and genotoxicity (Nos 2225–2228) were available. Data from short-term studies of toxicity on previously evaluated flavouring agents were used to support the safety evaluation of two of the additional flavouring agents in the group.

The Committee concluded that four of the five additional flavouring agents (Nos 2225–2228) in the group of aliphatic and aromatic amines and amides do not give rise to safety concerns at current estimated dietary exposures. For No. 2229, the Committee requires additional toxicological and/or dietary exposure information in order to complete the evaluation.

With respect to the four flavouring agents presented for re-evaluation (Nos 1595, 2005, 2010 and 2011), the Committee concluded that the Procedure still could not be applied to No. 1595 because of the concerns identified above. The Committee noted that No. 1595 is the flavouring agent with the highest poundage of the four flavouring agents presented for re-evaluation. Information that would assist in resolving the concerns would include data informing on the difference in response observed in kidneys of male and female rats in the comet

assay and on the potential of this compound to form reactive metabolites, as well as additional information on the kidney effects found at relatively low doses. For Nos 2005, 2010 and 2011, in the absence of data on these or structurally related flavouring agents, the Committee requires additional information in order to complete the evaluation.

An addendum to the monograph was prepared.

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4.1.3 Aliphatic secondary alcohols, ketones and related esters

Introduction

The Committee evaluated an additional six flavouring agents belonging to the group of aliphatic secondary alcohols, ketones and related esters (Nos 2216–

2221). These flavouring agents included two unsaturated secondary alcohols (Nos 2218 and 2220), one saturated secondary alcohol (No. 2221) and three unsaturated ketones (Nos 2216, 2217 and 2219). 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 63 other members of this group of flavouring agents at its fifty-ninth, sixty-ninth and seventy-third meetings (Annex 1, references *160*, *190* and *202*). The Committee concluded that all 63 flavouring agents were of no safety concern at estimated dietary exposures.

Two of the six flavouring agents considered at the current meeting – namely, 1,5-octadien-3-ol (No. 2218) and 3,5-undecadien-2-one (No. 2219) – have been reported to occur as natural components of green and black tea, fish oil, lean fish, oysters, scallops, brie, cooked chicken and chicken fat [1, 2].

Assessment of dietary exposure

The total annual volumes of production of the six flavouring agents belonging to the group of aliphatic secondary alcohols, ketones and related esters are approximately 407 kg in Europe, 1.1 kg in the USA and 0.1 kg in Japan [3]. Approximately 93% of the total annual volume of production in Europe is accounted for by one flavouring agent in this group – 1,5-octadien-3-ol (No. 2218).

Dietary exposures were estimated using the MSDI method and the SPET. The highest estimated dietary exposure for each flavouring agent is reported in Table 3. The estimated daily dietary exposure is highest for (±)-1-cyclohexylethanol (No. 2221) (3000 µg/day, the SPET value obtained from hard candy) [4]. For the other flavouring agents, dietary exposures as SPET or MSDI estimates range from 0.01 to 2000 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 secondary alcohols, ketones and related esters has previously been described in the monographs of the fifty-ninth and sixty-ninth meetings (Annex 1, references *161* and *191*). 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 six flavouring agents (Nos 2216–2221) to structural class II [5].

Table 3

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

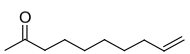
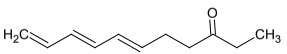
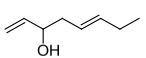
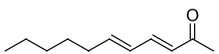
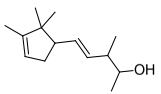
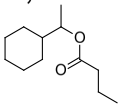
Flavouring agent	No.	CAS No. and structure	*Step A3*[d] Does estimated dietary exposure exceed the threshold of concern?	*Step A4* Is the flavouring agent or are its metabolites endogenous?	*Step A5*[e] Adequate margin of exposure for the flavouring agent or related substances?	Comments on predicted metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
Structural class II								
9-Decen-2-one	2216	35194-30-0 	Yes, SPET: 2 000	No	Yes. The NOAEL of 1 000 mg/kg bw per day (the highest dose tested) in a 28-day study in rats [6] is 30 000 times the estimated dietary exposure to No. 2216 when used as a flavouring agent.	Notes 1 and 2	–	No safety concern
Yuzunone	2217	1009814-14-5 	No, SPET: 15	NR	NR	Note 1	–	No safety concern
1,5-Octadien-3-ol	2218	83861-74-9 	No, SPET: 50	NR	NR	Notes 3 and 4	–	No safety concern
3,5-Undecadien-2-one	2219	68973-20-6 	No, SPET: 300	NR	NR	Notes 1 and 2	–	No safety concern
3-Methyl-5-(2,2,3-trimethylcyclopent-3-en-1-yl)pent-4-en-2-ol	2220	67801-20-1 	No, SPET: 300	NR	NR	Notes 2 and 3	–	No safety concern

Table 3 (continued)

Flavouring agent	No.	CAS No. and structure	*Step A3*[d] Does estimated dietary exposure exceed the threshold of concern?	*Step A4* Is the flavouring agent or are its metabolites endogenous?	*Step A5*[e] Adequate margin of exposure for the flavouring agent or related substances?	Comments on predicted metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
(±)-1-Cyclohexylethanol	2221	1193-81-3 	Yes, SPET: 3 000	No	Yes. The NOAEL of 300 mg/kg bw per day for the structurally related 1-cyclohexylethyl butyrate in a 28-day study in rats [7] is 6 000 times the estimated dietary exposure to No. 2221 when used as a flavouring agent.	Notes 2 and 3	1-Cyclohexylethyl butyrate 	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] Sixty-three flavouring agents in this group were previously evaluated by the Committee (Annex 1, references *160*, *190* and *202*).
[b] *Step 1*: All six flavouring agents are in structural class II.
[c] *Step 2*: All 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 II is 540 µg/day. All dietary exposure values are expressed in µg/day. The dietary exposure values listed represent the highest values calculated by either the SPET or MSDI method. The SPET gave the highest estimated dietary exposure in each case.
[e] The MOEs were calculated based on estimated dietary exposure calculated using the SPET.

Notes:
1. Ketone conjugated with glutathione and/or reduced to the secondary alcohol, which is then conjugated with glucuronic acid.
2. Methyl ketones or secondary alcohols that can be oxidized to methyl ketones can undergo alpha-hydroxylation and subsequent oxidation to form alpha-ketocarboxylic acids, which can undergo decarboxylation to yield carbon dioxide and simple aliphatic carboxylic acids.
3. Secondary alcohol conjugated with glucuronic acid.
4. Alpha, beta-unsaturated secondary alcohols may undergo conjugation with glutathione.

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

Step A3. The highest estimated dietary exposures for four of the flavouring agents (Nos 2217–2220) are below the threshold of concern (i.e. 540 μg/day for class II). The Committee therefore concluded that none of these four flavouring agents would pose a safety concern at current estimated dietary exposures. Two of the flavouring agents (Nos 2216 and 2221) have estimated dietary exposures greater than the threshold of concern (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 2216 and 2221) and their metabolites are not endogenous, and therefore their evaluations proceeded to step A5.

Step A5. For 9-decen-2-one (No. 2216), the NOAEL of 1000 mg/kg bw per day, the highest dose tested, obtained from a 28-day study in rats [6] provides an adequate MOE of 30 000 in relation to the estimated dietary exposure to No. 2216 (SPET = 2000 μg or 33 μg/kg bw per day) when used as a flavouring agent. The Committee therefore concluded that 9-decen-2-one (No. 2216) would not pose a safety concern at current estimated dietary exposures.

For (±)-1-cyclohexylethanol (No. 2221), the NOAEL of 300 mg/kg bw per day for the structurally related 1-cyclohexylethyl butyrate (CAS No. 63449-88-7) obtained from a 28-day study in rats [7] provides an adequate MOE of 6000 in relation to the highest estimated dietary exposure to No. 2221 (SPET = 3000 μg/day or 50 μg/kg bw per day) when used as a flavouring agent. The Committee therefore concluded that (±)-1-cyclohexylethanol (No. 2221) would not pose a safety concern at current estimated dietary exposures.

Table 3 summarizes the evaluations of the six flavouring agents belonging to this group of aliphatic secondary alcohols, ketones and related esters (Nos 2216–2221).

Consideration of combined intakes from use as flavouring agents

The six additional flavouring agents in this group of aliphatic secondary alcohols, ketones and related esters have low MSDI values (0.01–32 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.

Consideration of secondary components

One flavouring agent in this group (No. 2220) has a minimum assay value of less than 95% (see Annex 3). The secondary components are 6-(2,2,3-trimethylcyclopent-

3-en-1-yl)hex-5-en-3-ol (CAS No. 68480-05-7), present at 4–5%, and 3-methyl-5-(2,2,3-trimethylcyclopent-3-en-1-yl)pent-3-en-2-one (CAS No. 65113-95-3), present at 1–2%. These substances are structurally similar to No. 2220 and are considered not to present a safety concern at estimated dietary exposures from use of No. 2220 as a flavouring agent.

Conclusions

In the previous evaluations of flavouring agents in the group of aliphatic secondary alcohols, ketones and related esters, studies of acute toxicity, short-term toxicity and genotoxicity were available (Annex 1, references *161*, *191* and *203*). None of the 63 previously evaluated flavouring agents raised safety concerns.

For previously evaluated flavouring agents in this group, additional studies of acute toxicity (Nos 1151, 1152 and 2071), short-term toxicity (No. 1120), developmental toxicity (No. 1120) and genotoxicity (Nos 1129, 1136, 1150 and 1836) were available for this meeting. These additional data raised no safety concerns and supported the previous evaluations.

For the present evaluation of six flavouring agents that are additions to this group (Nos 2216–2221), studies of acute toxicity (No. 2216), short-term toxicity (Nos 2216 and 2220), genotoxicity (Nos 2216 and 2220) and reproductive toxicity (No. 2220) were available. Short-term studies of toxicity and studies of genotoxicity were available for 1-cyclohexylethyl butyrate, a substance structurally related to (±)-1-cyclohexylethanol (No. 2221). The Committee concluded that these six flavouring agents, which are additions to the group of aliphatic secondary alcohols, ketones and related esters evaluated previously, would not give rise to safety concerns at current estimated dietary exposures.

An addendum to the monograph was prepared.

References

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the Flavor Industry, Brussels, Belgium; 2008. Submitted to WHO by the International Organization for the Flavor Industry, Brussels, Belgium.

7. Stevens MW. Four week oral range finding study in rats. Unpublished report (study no. 401-030) from the International Research and Development Corporation, Mattawan, MI, USA. Unpublished report to the Research Institute for Fragrance Materials, Woodcliff Lake, NJ, USA; 1978. Submitted to WHO by the International Organization for the Flavor Industry, Brussels, Belgium.

4.1.4 Cinnamyl alcohol and related substances

Introduction

The Committee evaluated an additional five flavouring agents belonging to the group of cinnamyl alcohol and related substances (Nos 2211–2215). These flavouring agents included two esters (one with an additional aldehyde functional group: No. 2211; and one with an additional alcohol functional group: No. 2213), an aldehyde with a methylenedioxyphenyl functional group (No. 2212) and two acetals (Nos 2214 and 2215). 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 55 other members of this group of flavouring agents at its fifty-fifth meeting (Annex 1, reference *149*). The Committee concluded that all 55 flavouring agents in that group were of no safety concern at estimated dietary exposures.

One of the five flavouring agents considered at the current meeting, ethyl alpha-acetylcinnamate (No. 2211), has been reported to occur as a natural component of passion fruit juice [1].

Assessment of dietary exposure

The total annual volumes of production of the five flavouring agents belonging to the group of cinnamyl alcohol and related substances are approximately 32 kg in Europe, 0.1 kg in the USA and 31 kg in Japan [2].

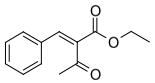
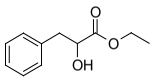
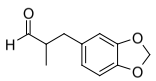
Dietary exposures were estimated using both the MSDI method and the SPET. The highest estimated dietary exposure for each flavouring agent is reported in Table 4. The estimated daily dietary exposure is highest for 3-(3,4-methylenedioxyphenyl)-2-methylpropanal (No. 2212) (3000 µg/day, the SPET value obtained from condiments and relishes) [3]. For the other flavouring agents, dietary exposures as SPET or MSDI estimates range from 0.01 to 180 µg/day, with the SPET yielding the highest estimate in each case.

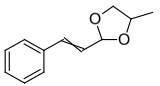
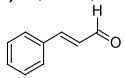
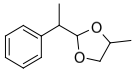
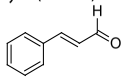
Absorption, distribution, metabolism and elimination

Information on the absorption, distribution, metabolism and elimination of flavouring agents belonging to the group of cinnamyl alcohol and related

Table 4

Summary of the results of the safety evaluations of cinnamyl alcohol and 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 A4* Is the flavouring agent or are its metabolites endogenous?	*Step A5*[e] Adequate margin of exposure for the flavouring agent or related substances?	*Follow-on from step B3* Are additional data available for the flavouring agent with an estimated dietary exposure exceeding the threshold of concern?	Comments on predicted metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
Structural class I									
Ethyl alpha-acetylcinnamate	2211	620-80-4 	A3: No, SPET: 30	NR	NR	NA	Note 1	–	No safety concern
Ethyl 2-hydroxy-3-phenylpropionate	2213	15399-05-0 	A3: No, SPET: 21	NR	NR	NA	Note 2	–	No safety concern
Structural class III									
3-(3,4-Methylenedioxyphenyl)-2-methylpropanal	2212	1205-17-0 	B3: Yes, SPET: 3 000	NA	NA	Yes. Some data are available; however, an appropriate repeated-dose toxicity study on No. 2212 or a closely related substance is not available.	Note 3	–	Additional data required to complete evaluation

Flavouring agent	No.	CAS No. and structure	*Step A3/B3*[d] Does estimated dietary exposure exceed the threshold of concern?	*Step A4* Is the flavouring agent or are its metabolites endogenous?	*Step A5*[e] Adequate margin of exposure for the flavouring agent or related substances?	*Follow-on from step B3* Are additional data available for the flavouring agent with an estimated dietary exposure exceeding the threshold of concern?	Comments on predicted metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
Cinnamaldehyde propyleneglycol acetal	2214	4353-01-9 	A3: Yes, SPET: 180	No	Yes. The NOAEL of 275 mg/kg bw per day for the structurally related cinnamaldehyde in a 14-week study in rats [5] is 92 000 times the estimated dietary exposure to No. 2214 when used as a flavouring agent.	NA	Note 4	Cinnamaldehyde (No. 656) 	No safety concern
2-Phenylpropanal propyleneglycol acetal	2215	67634-23-5 	Yes, SPET: 180	No	Yes. The NOAEL of 275 mg/kg bw per day for the structurally related cinnamaldehyde in a 14-week study in rats [5] is 92 000 times the estimated dietary exposure to No. 2215 when used as a flavouring agent.	NA	Note 5	Cinnamaldehyde (No. 656) 	No safety concern

NA: not applicable; NR: not required for evaluation because consumption of the flavouring agent was determined to be of no safety concern at step A3 of the Procedure

[a] Fifty-five flavouring agents in this group were previously evaluated by the Committee (Annex 1, reference *149*).
[b] *Step 1*: Two flavouring agents (Nos 2211 and 2213) are in structural class I. Three flavouring agents (Nos 2212, 2214 and 2215) are in structural class III.
[c] *Step 2*: Nos 2211, 2213, 2214 and 2215 can be predicted to be metabolized to innocuous products. No. 2212 cannot be predicted to be metabolized to innocuous products.
[d] The thresholds for human dietary exposure for structural classes I and III are 1800 and 90 µg/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 MSDI method. The SPET gave the highest estimated dietary exposure in each case.
[e] The MOEs were calculated based on estimated dietary exposure calculated using the SPET.

Notes:
1. Hydrolysed to ethanol and the corresponding alpha-keto carboxylic acid, which may undergo decarboxylation to form the ketone. The ketone can be metabolized to the corresponding secondary alcohol. Secondary alcohols typically form conjugates with glucuronic acid, which are readily eliminated in the urine.
2. Hydrolysed to ethanol and the corresponding alpha-hydroxy carboxylic acid, which may undergo oxidative decarboxylation, yielding benzoic acid. Benzoic acid is excreted as hippuric acid.
3. Aldehyde oxidized to the corresponding acid, which then undergoes beta-oxidation to its benzoic acid derivative. This is followed by conjugation with glycine to a hippuric acid derivative, which is excreted in the urine. The methylenedioxyphenyl functional group may be metabolized to *ortho*-dihydroxyphenyl, then to the *ortho*-quinone.
4. Hydrolysed to cinnamaldehyde and 1,2-propanediol. Cinnamaldehyde is oxidized to cinnamic acid, then further oxidized to benzoic acid. Benzoic acid is then conjugated with glycine to form hippuric acid, which is excreted in the urine. 1,2-Propanediol is metabolized to lactic acid and pyruvic acid.
5. Hydrolysed to the corresponding aldehyde and 1,2-propanediol. The aldehyde is further oxidized to its benzoic acid derivative, which is conjugated to glycine and/or glucuronic acid.

substances has previously been described in the monograph of the fifty-fifth meeting (Annex 1, reference *150*).

Esters of cinnamic acid and its saturated derivatives, such as ethyl alpha-acetyl cinnamate (No. 2211) and ethyl 2-hydroxy-3-phenylpropionate (No. 2213), are expected to be hydrolysed to the corresponding carboxylic acid and alcohol. However, because alpha and beta substituents larger than a methyl group have been shown to inhibit the beta-oxidation pathway, the hydrolytic product, alpha-acetyl cinnamic acid, is expected to be excreted unchanged or as the glucuronic acid conjugate. The acid hydrolysis product of ethyl 2-hydroxy-3-phenylpropionate (No. 2213) is an alpha-hydroxy acid, which is expected to undergo oxidative decarboxylation to form benzoic acid; the benzoic acid then undergoes conjugation with glycine to give hippuric acid, which is excreted in the urine. 3-(3,4-Methylenedioxyphenyl)-2-methylpropanal (No. 2212) may be oxidized to its corresponding acid and undergo beta-oxidation to its benzoic acid derivative, followed by conjugation with glycine to a hippuric acid derivative. The methylenedioxyphenyl functional group of No. 2212 may be metabolized to catechol and *ortho*-quinone intermediates.

The aromatic acetals cinnamaldehyde propyleneglycol acetal (No. 2214) and 2-phenylpropanal propyleneglycol acetal (No. 2215) are expected to readily hydrolyse, yielding the corresponding aldehydes and 1,2-propanediol. Following oxidation to their corresponding acids, 2-phenylpropionic acid is excreted in the urine as the glucuronic acid conjugate, and cinnamic acid is excreted in the urine as hippuric acid following beta-oxidation to benzoic acid and conjugation with glycine. 1,2-Propanediol is metabolized to lactic acid and pyruvic 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 agents, the Committee assigned two flavouring agents (Nos 2211 and 2213) to structural class I and three flavouring agents (Nos 2212, 2214 and 2215) to structural class III [4].

Step 2. Four of the flavouring agents (Nos 2211, 2213, 2214 and 2215) 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. 2212) 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 dietary exposures for each of the two flavouring agents in structural class I that are predicted to be metabolized to innocuous products (Nos 2211 and 2213) 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. The two flavouring agents in structural class III that are predicted to be metabolized to innocuous products (Nos 2214 and 2215) have estimated dietary exposures greater than the threshold of concern (i.e. 90 μg/day for class III). Accordingly, the evaluation of these flavouring agents proceeded to step A4.

Step A4. These flavouring agents (Nos 2214 and 2215) and their metabolites are not endogenous, and therefore their evaluations proceeded to step A5.

Step A5. For cinnamaldehyde propyleneglycol acetal (No. 2214), the NOAEL of 275 mg/kg bw per day for the structurally related substance cinnamaldehyde (No. 656) obtained from a 14-week study in rats [5] provides an adequate MOE of 92 000 in relation to the estimated dietary exposure to No. 2214 (SPET = 180 μg/day or 3 μg/kg bw per day) when used as a flavouring agent. The Committee therefore concluded that cinnamaldehyde propyleneglycol acetal (No. 2214) would not pose a safety concern at current estimated dietary exposures.

For 2-phenylpropanal propyleneglycol acetal (No. 2215), the NOAEL of 275 mg/kg bw per day for the structurally related substance cinnamaldehyde (No. 656) obtained from a 14-week study in rats [5] provides an adequate MOE of 92 000 in relation to the estimated dietary exposure to No. 2215 (SPET = 180 μg/day or 3 μg/kg bw per day) when used as a flavouring agent. The Committee therefore concluded that 2-phenylpropanal propyleneglycol acetal (No. 2215) would not pose a safety concern at current estimated dietary exposures.

Step B3. The highest estimated daily dietary exposure for the flavouring agent in structural class III that is not predicted to be metabolized to innocuous products (No. 2212) is greater than the threshold of concern (i.e. 90 μg/day for class III). Accordingly, data must be available on the substance or a closely related substance in order to perform a safety evaluation.

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

In accordance with the Procedure, additional data were evaluated for 3-(3,4-methylenedioxyphenyl)-2-methylpropanal (No. 2212), as its estimated dietary exposure exceeded the threshold of concern for structural class III (90 μg/day). Studies of acute toxicity, genotoxicity, and reproductive and developmental toxicity were available. Oral LD_{50} values in mice and rats were reported as 1035 and 3561 mg/kg bw, respectively [6, 7]. Reverse mutation assays in *Salmonella typhimurium* and *Escherichia coli*, with and without exogenous metabolic activation, were negative [8]. An in vitro chromosome aberration assay in Chinese hamster ovary cells was positive for structural chromosome aberrations

but negative for numerical chromosome aberrations [9]. An in vivo study of micronucleus induction in bone marrow of mice administered No. 2212 at up to 725 mg/kg bw by intraperitoneal injection was negative [7]. No. 2212 contains a methylenedioxyphenyl functional group, and some substances with this functional group (e.g. safrole) exhibit genotoxicity and have shown carcinogenic activity in rodent studies [10] (Annex 1, reference *57*). However, No. 2212 does not contain the alkenylbenzene functional group that is present in safrole, and this functional group is considered to be essential for the genotoxic activity of safrole [11]. The Committee therefore concluded that No. 2212 is unlikely to present a genotoxicity concern.

In a 14-day screening study of reproductive toxicity in male rats, there were no adverse effects of No. 2212 on sperm parameters or reproductive organs at a gavage dose of 1000 mg/kg bw per day, the only dose tested [12]. In a developmental toxicity study, gavage administration of No. 2212 to pregnant rats was associated with clinical signs of toxicity, reductions in body weight gain and absolute and relative feed consumption at the high dose of 250 mg/kg bw per day. The maternal NOAEL for 3-(3,4-methylenedioxyphenyl)-2-methylpropanal (No. 2212) was therefore considered to be the middle dose (125 mg/kg bw per day). There were no treatment-related effects on pregnancy parameters, and all fetuses appeared normal upon examination. The NOAEL for effects on development was therefore 250 mg/kg bw per day, the highest dose tested [13].

Concerns have been raised regarding the potential general toxicity (e.g. hepatotoxicity) of substances containing the methylenedioxyphenyl functional group [14]. No appropriate repeated-dose toxicity study on No. 2212 or a closely related substance was available that would be suitable to support the safety evaluation of this flavouring agent. Therefore, the Committee concluded that additional data are required to complete the evaluation of 3-(3,4-methylenedioxyphenyl)-2-methylpropanal (No. 2212).

Table 4 summarizes the evaluations of the five flavouring agents belonging to this group of cinnamyl alcohol and related substances (Nos 2211–2215).

Consideration of combined intakes from use as flavouring agents

The five additional flavouring agents in this group of cinnamyl alcohol and related substances have low MSDI values (0.01–4 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.

Consideration of secondary components

One flavouring agent in this group (No. 2214) has a minimum assay value of less than 95% (see Annex 3). The major secondary component, cinnamaldehyde (No. 656), present at 4–5%, is considered not to present a safety concern at estimated dietary exposures from use of No. 2214 as a flavouring agent.

Consideration of additional data on previously evaluated flavouring agents

For the previously evaluated flavouring agents in this group, additional studies on absorption and metabolism (Nos 656 and 680), acute toxicity (No. 680), short-term toxicity (No. 656), long-term toxicity (No. 656), genotoxicity (Nos 650, 656, 657, 658, 668, 671, 680, 683, 686 and 688) and reproductive toxicity (Nos 680 and 686) were available for this meeting.

Reproductive toxicity studies in male rats and rabbits with 3-(*p*-isopropylphenyl) propionaldehyde (No. 680) showed increases in the incidence of abnormal sperm and adverse effects on the testes, with NOAELs of 25 mg/kg bw per day in rats and 100 mg/kg bw per day in rabbits [15, 16]. No. 680 is the only member of the group of cinnamyl alcohol and related substances evaluated to date that possesses a *para*-isopropyl group. The adverse effects on male reproduction parameters may be related to the presence of this functional group. No. 680 belongs to structural class I, and an MSDI of 0.1 μg/day (0.002 μg/kg bw per day) was calculated at the fifty-fifth meeting (Annex 1, reference *149*). The NOAEL of 25 mg/kg bw per day from the rat reproductive toxicity study [15] provides an adequate MOE of 15 million in relation to the MSDI of 0.1 μg/day. The Committee noted that the most recent annual volume of production of No. 680 as a flavouring agent is reported as 0 kg in Europe, the USA and Japan [3].

The Committee concluded that the new toxicity data on previously evaluated flavouring agents in this group raised no safety concerns.

Conclusions

In the previous evaluation of flavouring agents in this group of cinnamyl alcohol and related substances, studies of acute toxicity, short-term and long-term toxicity, genotoxicity, and reproductive and developmental toxicity were available (Annex 1, reference *150*). None of the 55 previously evaluated flavouring agents raised safety concerns.

For several previously evaluated flavouring agents in this group, additional toxicity data were available for this meeting. The additional data raised no safety concerns and supported the previous evaluation.

For the present evaluation of five flavouring agents that are additions to this group (Nos 2211–2215), studies of acute toxicity (Nos 2212 and 2215), genotoxicity (Nos 2212 and 2215) and reproductive and developmental toxicity

(No. 2212) were available. The Committee concluded that four of these flavouring agents (Nos 2211, 2213, 2214 and 2215) would not give rise to safety concerns at current estimated dietary exposures. For No. 2212, repeated-dose toxicity data on the flavouring agent or a closely related substance are required to complete the safety evaluation.

An addendum to the monograph was prepared.

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4.1.5 Tetrahydrofuran and furanone derivatives

Introduction

The Committee evaluated five additional flavouring agents belonging to the group of tetrahydrofuran and furanone derivatives. The additional flavouring agents (Nos 2230–2234) are all substituted furanones. The evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents (Annex 1, reference *131*). None of these flavouring agents has previously been evaluated by the Committee.

The Committee previously evaluated 18 other members of this group of flavouring agents at its sixty-third meeting (Annex 1, reference *173*). The Committee concluded that all 18 flavouring agents were of no safety concern at estimated dietary exposures.

Three of the five flavouring agents in this group (Nos 2230–2232) have been reported to occur naturally in foods. They have been detected in, for example, coffee, mango, passion fruit and juice, wheat bread, wild rice and peanuts [1].

Assessment of dietary exposure

The total annual volume of production of the five flavouring agents belonging to the group of tetrahydrofuran and furanone derivatives is approximately 14 kg in Europe, 14 kg in the USA and 23 kg in Japan [2, 3]. The entire volume (100%) of the annual production in Europe and more than 90% of the annual production volume in Japan are accounted for by No. 2230. In the USA, No. 2230 accounts for over 70% of the annual production volume, followed by 29% accounted for by No. 2231.

Dietary exposures were estimated using both the MSDI method and the SPET, with the highest values reported in Table 5. The estimated dietary exposure is highest for Nos 2230–2232 and 2234 (600 µg/day, SPET value for gelatines and puddings and milk products). For the remaining flavouring agent, No. 2233, the SPET also yielded the highest estimated dietary exposure estimate.

Absorption, distribution, metabolism and elimination

Information on the absorption, distribution, metabolism and elimination of flavouring agents belonging to the group of tetrahydrofuran and furanone derivatives has previously been described (Annex 1, reference *174*). No additional information was available for this meeting.

The ether- and ester-substituted furanone derivatives in this additional group of five flavouring agents (Nos 2231 and 2233) are predicted to be readily oxidized or hydrolysed, resulting in a hydroxyl-substituted furanone. Based on metabolic data from 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (DMHF; No. 1446), the hydroxyl-substituted furanone will be rapidly conjugated with glucuronic acid and eliminated via the urine [4].

The alkyl-substituted furanone derivatives (Nos 2230, 2232 and 2234) need to undergo ring or side-chain oxidation, or keto-reduction by cytosolic carbonyl reductase, before conjugation of the resulting alcohol and excretion 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. 2230) to structural class II and four flavouring agents (Nos 2231–2234) to structural class III [5].

Step 2. Two of the flavouring agents (Nos 2231 and 2233) 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 agents (Nos 2230, 2232 and 2234) in this group cannot be predicted to be metabolized to innocuous products. Therefore, the evaluation of these flavouring agents proceeded via the B-side of the Procedure.

Step A3. The highest estimated daily dietary exposures for each of the two flavouring agents in structural class III that are predicted to be metabolized to innocuous products (Nos 2231 and 2233) are above the threshold of concern (i.e. 90 µg/day for class III). Accordingly, the evaluation of these flavouring agents proceeded to step A4.

Step A4. These flavouring agents (Nos 2231 and 2233) and their metabolites are not endogenous, and therefore their evaluations proceeded to step A5.

Table 5

Summary of the results of the safety evaluations of tetrahydrofuran and furanone derivatives used as flavouring agents[a,b,c]

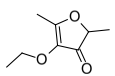
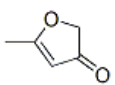
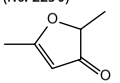
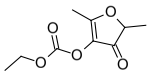
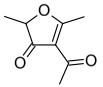
Flavouring agent	No.	CAS No. and structure	*Step A3/B3*[d] Does estimated dietary exposure exceed the threshold of concern?	*Step A4* Is the flavouring agent or are its metabolites endogenous?	*Step A5*[e] Adequate margin of exposure for the flavouring agent or related substances?	*Follow-on from step B3*[e] Are additional data available for the flavouring agent with an estimated dietary exposure exceeding the threshold of concern?	Comments on predicted metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
Structural class II									
2,5-Dimethyl-3(2*H*)-furanone	2230	14400-67-0 	B3: Yes, SPET: 600	NR	NR	Yes. No. 2230 is non-genotoxic in bacteria and in mammalian cells in vitro, and the NOAEL of 15 mg/kg bw per day in a 90-day study in rats [9] is 1 500 times the estimated dietary exposure to No. 2230 when used as a flavouring agent.	Note 1	–	No safety concern
Structural class III									
2,5-Dimethyl-4-ethoxy-3(2*H*)-furanone	2231	65330-49-6 	A3: Yes, SPET: 600	No	Yes. The NOAEL of 200 mg/kg bw per day for the structurally related 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (No. 1446) in a 2-year study in rats [6] is 20 000 times the estimated dietary exposure to No. 2231 when used as a flavouring agent.	NR	Note 2	4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone (No. 1446) 	No safety concern
5-Methyl-3(2*H*)-furanone	2232	3511-32-8 	B3: Yes, SPET: 600	NR	NR	Yes, for the structurally related 2,5-dimethyl-3(2*H*)-furanone (No. 2230). This compound is non-genotoxic in bacteria and in mammalian cells in vitro, and the NOAEL of 15 mg/kg bw per day in a 90-day study in rats [9] is 1 500 times the estimated dietary exposure to No. 2232 when used as a flavouring agent.	Note 1	2,5-Dimethyl-3(2*H*)-furanone (No. 2230) 	No safety concern

Table 5 (continued)

Flavouring agent	No.	CAS No. and structure	*Step A3/B3*[d] Does estimated dietary exposure exceed the threshold of concern?	*Step A4* Is the flavouring agent or are its metabolites endogenous?	*Step A5*[e] Adequate margin of exposure for the flavouring agent or related substances?	*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	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
Ethyl 2,5-dimethyl-3-oxo-4(2*H*)-furyl carbonate	2233	39156-54-2 	A3: Yes, SPET: 200	No	Yes. The NOAEL of 200 mg/kg bw per day for the structurally related 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (No. 1446) in a 2-year study in rats [6] is 67 000 times the estimated dietary exposure to No. 2231 when used as a flavouring agent.	NR	Note 3	4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone (No. 1446) 	No safety concern
4-Acetyl-2,5-dimethyl-3(2*H*)-furanone	2234	36871-78-0 	B3: Yes, SPET: 600	NR	NR	No	Notes 1 and 4	–	Additional data required to complete evaluation

NR: not relevant

[a] Eighteen flavouring agents in this group were previously evaluated by the Committee (Annex 1, reference *173*).
[b] *Step 1*: One flavouring agent (No. 2230) is in structural class II, and four flavouring agents (Nos 2231–2234) are in structural class III.
[c] *Step 2*: Nos 2231 and 2233 can be predicted to be metabolized to innocuous products. Nos 2230, 2232 and 2234 cannot be predicted to be metabolized to innocuous products.
[d] The thresholds for human dietary exposure for structural classes II and III are 540 and 90 µg/day, respectively. 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. Ring or side-chain hydroxylation followed by conjugation with glucuronic acid and excretion in the urine.
2. The ether group is readily oxidized, and the resulting furanone is conjugated with glucuronic acid and excreted in the urine.
3. The carbonate group is readily hydrolysed, and the resulting furanone is conjugated with glucuronic acid and excreted in the urine.
4. Keto-reduction by cytosolic carbonyl reductase, followed by conjugation with glucuronic acid and excretion in the urine.

Step A5. For 2,5-dimethyl-4-ethoxy-3(2*H*)-furanone (No. 2231), which is expected to be readily oxidized to DMHF (No. 1446), the NOAEL of 200 mg/kg bw per day for DMHF obtained in a 2-year toxicity study in rats [6] provides an adequate MOE of 20 000 in relation to the estimated dietary exposure to No. 2231 (SPET = 600 μg/day or 10 μg/kg bw per day) when used as a flavouring agent. The Committee therefore concluded that No. 2231 would not pose a safety concern at current estimated dietary exposures.

For ethyl 2,5-dimethyl-3-oxo-4(2*H*)-furyl carbonate (No. 2233), which is expected to be readily hydrolysed to DMHF (No. 1446), the NOAEL of 200 mg/kg bw per day for DMHF obtained in a 2-year toxicity study in rats [6] provides an adequate MOE of approximately 67 000 in relation to the estimated dietary exposure to No. 2233 (SPET = 200 μg/day or 3 μg/kg bw per day) when used as a flavouring agent. The Committee therefore concluded that No. 2233 would not pose a safety concern at current estimated dietary exposures.

Step B3. The highest estimated dietary exposure for the flavouring agent in structural class II (No. 2230) is above the threshold of concern (i.e. 540 μg/day for class II). The highest estimated dietary exposures for the flavouring agents in structural class III (Nos 2232 and 2234) are also above the threshold of concern (i.e. 90 μg/day for class III). Accordingly, data must be available on these flavouring agents or closely related substances 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 Nos 2230, 2232 and 2234, as their estimated dietary exposures exceeded the threshold of concern for structural class II (540 μg/day; No. 2230) or structural class III (90 μg/day; Nos 2232 and 2234).

For 2,5-dimethyl-3(2*H*)-furanone (No. 2230), a 90-day toxicity study and two in vitro genotoxicity studies were available. The flavouring agent was negative for bacterial mutagenicity with and without an exogenous activation system [7] and for induction of micronuclei in human peripheral blood lymphocytes [8]. The NOAEL of 15 mg/kg bw per day in a 90-day toxicity study in rats [9] provides an adequate MOE of 1500 in relation to the estimated dietary exposure to No. 2230 (SPET = 600 μg/day or 10 μg/kg bw per day) when used as a flavouring agent. The Committee concluded that, on the basis of all of the available evidence, No. 2230 would not pose a safety concern at current estimated dietary exposures.

For 5-methyl-3(2*H*)-furanone (No. 2232), no substance-specific data were available. However, the NOAEL of 15 mg/kg bw per day for the structurally related substance 2,5-dimethyl-3(2*H*)-furanone (No. 2230) in a 90-day toxicity study in rats [9] provides an adequate MOE of 1500 in relation to the estimated

dietary exposure to No. 2232 (SPET = 600 µg/day or 10 µg/kg bw per day) when used as a flavouring agent. The Committee therefore concluded that No. 2232 would not pose a safety concern at current estimated dietary exposures.

For 4-acetyl-2,5-dimethyl-3(2*H*)-furanone (No. 2234), only two in vitro genotoxicity studies were available. The flavouring agent was negative for bacterial mutagenicity with and without an exogenous activation system [10] and for induction of micronuclei in human peripheral blood lymphocytes [11, 12]. In the absence of a study identifying a NOAEL for this or a closely related flavouring agent, the Committee concluded that additional data are required to complete the evaluation.

Table 5 summarizes the evaluations of the five additional flavouring agents (Nos 2230–2234) in the group of tetrahydrofuran and furanone derivatives.

Consideration of combined intakes from use as flavouring agents

The five additional flavouring agents in the group of tetrahydrofuran and furanone derivatives have low MSDIs (0.01–6 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. 2233) has a minimum assay value of less than 95% (see Annex 3). The major secondary component in No. 2233, present at 5–6%, is 2,5-dimethylfuran-3,4-diyl diethyl bis(carbonate). This compound is predicted to undergo rapid hydrolysis of the carbonate moieties to form the unstable intermediate of 2,5-dimethylfuran-3,4-diol, which will rapidly oxidize under acidic conditions to form 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (No. 1446). This flavouring agent was previously evaluated by the Committee (Annex 1, reference *173*) and considered not to present a safety concern at estimated dietary exposures.

Conclusion

In the previous evaluation of flavouring agents in this group of tetrahydrofuran and furanone derivatives, acute toxicity studies, short-term studies of toxicity, long-term studies of toxicity and carcinogenicity, and genotoxicity studies were available (Annex 1, reference *174*). None of the 18 previously evaluated flavouring agents raised safety concerns.

For the present evaluation, studies of short-term toxicity (No. 2230) and genotoxicity (Nos 2230 and 2234) were available for the flavouring agents in this group. For previously evaluated flavouring agents in this group, studies of

acute toxicity (Nos 1443, 1448, 1449, 1452 and 1456), short-term toxicity (Nos 1443 and 1452), genotoxicity (Nos 1443, 1449 and 1456) and reproductive and developmental toxicity (Nos 1443 and 1446) were available. The studies available for the present evaluation support the previous safety evaluations.

The Committee concluded that four of these five flavouring agents (Nos 2230–2233), which are additions to the group of tetrahydrofuran and furanone derivatives evaluated previously, do not give rise to safety concerns at current estimated dietary exposures. For No. 2234, the Committee requires additional toxicological and/or dietary exposure information in order to complete the evaluation.

An addendum to the monograph was prepared.

References

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4.2 Specifications of identity and purity of flavouring agents

4.2.1 New specifications

The Committee received information related to specifications for 23 of the 24 new flavouring agents from the call for data for the present meeting. Full specifications were prepared for the 23 new flavouring agents for which data were provided. The specifications prepared for three of the flavouring agents, 4-acetyl-2,5-dimethyl-3(2*H*)-furanone (No. 2234) (see section 4.1.5), 3-(3,4-methylenedioxyphenyl)-2-methylpropanal (No. 2212) (see section 4.1.4) and *N*-ethyl-5-methyl-2-(methylethenyl)cyclohexanecarboxamide (No. 2229) (see section 4.1.2), include a statement that the safety evaluation for the flavouring agents had not been completed.

Four flavouring agents for which full specifications currently exist (Nos 1595, 2005, 2010 and 2011) were considered by the Committee for toxicological re-evaluation at the current meeting (see section 4.1.2). The toxicological re-evaluation could not be completed at the current meeting, as additional information is still required. Therefore, the statement currently contained in the specifications indicating that the safety evaluation had not been completed will be maintained.

4.2.2 Revised specifications

The Committee received information in support of the revision of six full flavouring agent specifications that were on the agenda of the present meeting.

The Committee revised the specifications for 3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one (No. 1114) by changing the assay minimum from greater than 98% as the *cis* isomer to greater than 95% as a sum of isomers, revising the ranges for refractive index and specific gravity, and introducing new information on the isomeric composition of the flavouring agent.

The Committee revised the specifications for 6,10-dimethyl-5,9-undecadien-2-one (No. 1122) by indicating that the assay minimum was for a sum of isomers, changing the CAS number, revising the information for solubility in ethanol, revising the ranges for refractive index and specific gravity,

and introducing new information on the isomeric composition of the flavouring agent.

The Committee revised the specifications for 3-ammonium isovalerate (No. 1203) by correcting the molecular weight and chemical formula and revising the melting point range for the flavouring agent.

The Committee revised the specifications for theaspirane (No. 1238) by lowering the assay minimum from greater than 97% (sum of stereoisomers) to greater than 85% (sum of stereoisomers), revising the ranges for refractive index and specific gravity, and introducing new information on the isomeric composition and secondary components of the flavouring agent. Five secondary components were evaluated at the current meeting. The Cramer structural classes were determined and the exposure estimated based on the MSDI exposure estimate for theaspirane (No. 1238) determined at the sixty-first JECFA (Annex 1, reference *166*) and the percentage of the secondary component present in the flavouring. All estimated exposures were well below the respective class thresholds. It was therefore concluded that the five secondary components were of no safety concern at current estimated levels of exposure.

The Committee revised the specifications for alpha-bisabolol (No. 2031) by changing the assay minimum from greater than 93% to greater than 95% as a sum of isomers, adding a second CAS number, revising the ranges for refractive index and specific gravity, clarifying the range of the secondary component, and introducing new information on the isomeric composition of the flavouring agent.

The Committee revised the specifications for glutamyl-valyl-glycine (No. 2123) by lowering the assay minimum from greater than 99% to greater than 95% for the flavouring agent.

5. Future work and recommendations

General considerations

Revision of the Procedure for the Safety Evaluation of Flavouring Agents

The Committee recommended that the revised Procedure for the Safety Evaluation of Flavouring Agents should be applied in its future evaluations.

Approach for prioritizing flavouring agents for re-evaluation

The Committee reiterated the need for the development of an approach, including a prioritization process, for the re-evaluation of flavouring agents based on all available toxicological data and updated dietary exposure estimates.

Replacement of packed column gas chromatographic methods in the specifications monographs

The Committee recommended that the FAO JECFA Secretariat establish a process to identify the food additive specifications monographs containing packed column gas chromatographic methods and request suitable methods (through a call for data), in order for the Committee to replace these methods in the specifications monographs.

Revision of the FAO JECFA Monographs 1, Combined Compendium of Food Additive Specifications, Volume 4

The Committee recommended that the FAO JECFA Secretariat establish a process for the revision of FAO JECFA Monographs 1, Combined Compendium of Food Additive Specifications, Volume 4.

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

The Committee recommended that all additives for use in infant formula be reviewed for lead levels in the specifications.

Limits for arsenic in specifications of food additives for use in infant formula

The Committee recommended that all additives for use in infant formula be reviewed for arsenic levels in the specifications.

Use of chloroform as solvent in the test methods associated with specifications monographs for synthetic colours

The Committee recommended the development of analytical methods with suitable replacement solvent(s), in order to replace chloroform, in the future.

General inclusion of infrared spectra

The Committee recommended that all future specifications for new flavouring agents contain a high-quality readable infrared spectrum in the data submission.

Inclusion of chemical structures in the JECFA flavourings database

The Committee recommended that chemical structures be included in the JECFA flavourings database.

Specific food additives (other than flavouring agents)

Carob bean gum

The Committee concluded that the available information is not sufficient for the evaluation of carob bean gum for use in infant formula at the proposed use level and requested toxicological data on neonatal animals, adequate to evaluate the safety for use in infant formula, to complete the evaluation.

The Committee noted that the sponsor also identified a cold-soluble carob bean gum for use in infant formula. However, no information was provided on the manufacturing and composition of the product, and the Committee was unclear which product is used in infant formula and formula for special medical purposes intended for infants.

Cassia gum

The Committee noted that cassia gum can be obtained from a number of companies and requested information on validated methods of analysis currently in use by providers of cassia gum. The methods submitted should contain details of the use of standard (reference) materials, the extraction efficiency of the initial steps, the recovery of the analytes in question, performance data and the results of the analysis of several batches of the material in commerce.

The tentative specifications will be withdrawn unless the requested information is submitted **before 31 December 2017**.

Citric and fatty acid esters of glycerol (CITREM)

The Committee recommended that data be submitted for the replacement of the packed column gas chromatography test method for the determination of total citric acid with a suitable method using a capillary/wide-bore column for consideration at a future meeting.

Lutein esters from Tagetes erecta

The Committee at its seventy-ninth meeting 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. The current Committee was not able to consider this aspect in detail and recommended that this be taken up at a future meeting.

Modified starches

The Committee prepared tentative specifications for the following 13 modified starches and requires the following information for the removal of the tentative status:

Modified starch	Information required on
Dextrin roasted starch (INS No. 1400)	• A suitable method for the Dispersion or Reducing Sugars Distinguishing Test
Acid treated starch (INS No. 1401)	• A suitable method for the Dispersion or Reducing Sugars Distinguishing Test
Alkaline treated starch (INS No. 1402)	• A suitable method for the Dispersion or Reducing Sugars Distinguishing Test
Bleached starch (INS No. 1403)	• Typical levels of residual reagents or by-products
Enzyme-treated starch (INS No. 1405)	• A suitable method for the Dispersion or Reducing Sugars Distinguishing Test
Monostarch phosphate (INS No. 1410)	• A suitable test for identification of the phosphate groups
Distarch phosphate (INS No. 1412)	• A suitable test for identification of the phosphate groups and of crosslinking
Phosphated distarch phosphate (INS No. 1413)	• A suitable test for identification of the phosphate groups and of crosslinking
Acetylated distarch phosphate (INS No. 1414)	• A suitable test for identification of the phosphate groups and of crosslinking
Acetylated distarch adipate (INS No. 1422)	• A suitable test for identification of the adipate groups • Levels of free adipic acid
Hydroxypropyl starch (INS No. 1440)	• A suitable method for the determination of propylene chlorohydrin
Hydroxypropyl distarch phosphate (INS No. 1442)	• A suitable method for the determination of propylene chlorohydrin • A suitable test for identification of the phosphate groups
Starch sodium octenyl succinate (INS No. 1450)	• A suitable test for identification of octenylsuccinate groups

The Committee recommended that the call for data also include method of manufacture for each of the 16 modified starches. The missing data are required **by 31 December 2017**.

Rosemary extract

The Committee made the ADI temporary pending the submission of studies to elucidate the potential developmental and reproductive toxicity of the rosemary extract under consideration. The temporary ADI will be withdrawn if the required data are not provided **by the end of 2018**.

The Committee prepared tentative specifications and requested validation information on the method for determination of residual solvents **by the end of 2018**.

The Committee requested that data on typical use levels in foods be provided **by the end of 2018** in order to refine the dietary exposure estimates.

Steviol glycosides

The specifications were made tentative pending submission of the following information **by 31 December 2017**:

- method of assay to replace the existing method and including as many steviol glycosides as possible (at least those listed in Appendix 1 of the specifications) in steviol glycoside mixtures, along with supporting validation information and chromatograms;
- analytical results from a minimum of five batches for commercial samples, including supporting chromatograms.

Flavouring agents

Aliphatic and aromatic amines and amides

The Committee concluded that the concerns previously expressed by the Committee at its sixty-ninth meeting as to in vivo genotoxicity and how to address the kidney effects and identify a NOAEL had not been sufficiently addressed and that the Procedure for the Safety Evaluation of Flavouring Agents still could not be applied to 2-isopropyl-*N*,2,3-trimethylbutyramide (No. 1595). Information that would assist in resolving the concerns would include data informing on the difference in response observed in the kidney of male and female rats in the comet assay and on the potential of this compound to form reactive metabolites, as well as additional information on the kidney effects found at relatively low doses.

For *N*-ethyl-2,2-diisopropylbutanamide (No. 2005), *N*-(2-hydroxyethyl)-2,3-dimethyl-2-isopropylbutanamide (No. 2010) and *N*-(1,1-dimethyl-2-hydroxyethyl)-2,2-diethylbutanamide (No. 2011), NOAELs for these flavouring agents or structurally related substances were not available. Although No. 1595 is structurally related, the Committee concluded that No. 1595 could not be evaluated using the Procedure, and therefore this flavouring agent was not suitable to support the evaluation of these three flavouring agents. Therefore, for these three flavouring agents, the Committee concluded that additional data would be necessary to complete the evaluation.

For some previously evaluated flavouring agents in this group, additional toxicity data were available for this meeting. For *N*-isobutyl (*E*,*E*)-2,4-decadienamide (No. 1598) and (2*E*,6*E*/*Z*,8*E*)-*N*-(2-methylpropyl)-2,6,8-decatrienamide (No. 2077), the new studies resulted in lower NOAELs. In light of general considerations on the Procedure for the Safety Evaluation of Flavouring Agents and the need for an approach for re-evaluation in light of new data (see above), the Committee recommended re-evaluation of these two flavouring agents at a future meeting.

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 six flavouring agents (Nos 2005, 2010, 2011, 2212, 2229 and 2234).

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Annex 1

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112. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/6, 1994.

113. Evaluation of certain veterinary drug residues in food (Forty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 855, 1995, and corrigendum.

114. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 34, 1995.

115. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/7, 1995.

116. Evaluation of certain food additives and contaminants (Forty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 859, 1995.

117. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 35, 1996.

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119. Evaluation of certain veterinary drug residues in food (Forty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 864, 1996.

120. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 36, 1996.

121. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/8, 1996.

122. Evaluation of certain food additives and contaminants (Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 868, 1997.

123. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 37, 1996.

124. Compendium of food additive specifications, addendum 4. FAO Food and Nutrition Paper, No. 52, Add. 4, 1996.

125. Evaluation of certain veterinary drug residues in food (Forty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 876, 1998.

126. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 38, 1996.

127. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/9, 1997.

128. Evaluation of certain veterinary drug residues in food (Forty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 879, 1998.

129. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 39, 1997.

130. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/10, 1998.

131. Evaluation of certain food additives and contaminants (Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 884, 1999.

132. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 40, 1998.

133. Compendium of food additive specifications: addendum 5. FAO Food and Nutrition Paper, No. 52, Add. 5, 1997.

134. Evaluation of certain veterinary drug residues in food (Fiftieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 888, 1999.

135. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 41, 1998.

136. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/11, 1999.

137. Evaluation of certain food additives (Fifty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 891, 2000.

138. Safety evaluation of certain food additives. WHO Food Additives Series, No. 42, 1999.

139. Compendium of food additive specifications, addendum 6. FAO Food and Nutrition Paper, No. 52, Add. 6, 1998.

140. Evaluation of certain veterinary drug residues in food (Fifty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 893, 2000.

141. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 43, 2000.

142. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/12, 2000.

143. Evaluation of certain food additives and contaminants (Fifty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 896, 2000.

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

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

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

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

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

152. Evaluation of certain mycotoxins in food (Fifty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 906, 2002.

153. Safety evaluation of certain mycotoxins in food. WHO Food Additives Series, No. 47/FAO Food and Nutrition Paper, No. 74, 2001.

154. Evaluation of certain food additives and contaminants (Fifty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 909, 2002.

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

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

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

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

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

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

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

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

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

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

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

166. Evaluation of certain food additives and contaminants (Sixty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 922, 2004.

167. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 52, 2004.

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

169. Evaluation of certain veterinary drug residues in food (Sixty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 925, 2004.

170. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/16, 2004.

171. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 53, 2005.

172. Compendium of food additive specifications: addendum 12. FAO Food and Nutrition Paper, No. 52, Add. 12, 2004.

173. Evaluation of certain food additives (Sixty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 928, 2005.

174. Safety evaluation of certain food additives. WHO Food Additives Series, No. 54, 2005.

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

176. Evaluation of certain food contaminants (Sixty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 930, 2005.

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

178. Evaluation of certain food additives (Sixty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 934, 2006.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

214. 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.

215. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 68, 2013.

216. Compendium of food additive specifications. FAO JECFA Monographs 14, 2013.

217. 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.

218. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 69, 2014.

219. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs 15, 2014.

220. Evaluation of certain food additives (Seventy-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 990, 2015.

221. Safety evaluation of certain food additives. WHO Food Additives Series, No. 70, 2015.

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

223. Evaluation of certain food additives and contaminants (Eightieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 995, 2016.

224. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 71, 2015.

225. Compendium of food additive specifications. FAO JECFA Monographs 17, 2015.

226. Evaluation of certain veterinary drug residues in food (Eighty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 997, 2016.

227. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 72, 2016.

228. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs 18, 2016.

229. Safety evaluation of certain food additives and contaminants. Supplement 1: Non-dioxin-like polychlorinated biphenyls. WHO Food Additives Series, No. 71-1, 2016.

Annex 2

Toxicological information and information on specifications

Food additives evaluated toxicologically and assessed for dietary exposure

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions
Allura Red AC	R[a]	The Committee concluded that the new data do not give reason to revise the ADI and confirmed the ADI of 0–7 mg/kg body weight (bw). The Committee noted that the range of estimated dietary exposures to Allura Red AC for children based on reported or industry use data were below the upper bound of the ADI and concluded that dietary exposure to Allura Red AC for children and all other age groups does not present a health concern.
Carob bean gum	R[b]	**The Committee concluded that the available studies are not sufficient for the evaluation of carob bean gum for use in infant formula at the proposed use level.**[c] The Committee requests toxicological data from studies in neonatal animals, adequate to evaluate the safety for use in infant formula, to complete the evaluation.
Lutein esters from *Tagetes erecta*	R[d]	**The Committee removed the temporary designation from the ADI "not specified"**[e] (because the tentative status of the specifications was removed) **and established an ADI "not specified"** for lutein esters from *Tagetes erecta*.
Octenyl succinic acid (OSA)–modified gum arabic	R[f]	**The Committee removed the temporary designation from the ADI "not specified"**[e] **and established an ADI "not specified"** for OSA-modified gum arabic. The Committee confirmed the validity of the dietary exposure estimate for risk assessment purposes set at a previous meeting.
Pectin	R[g]	The no-observed-adverse-effect level (NOAEL) in a previously evaluated neonatal pig study was recalculated to be 1049 mg/kg bw per day using measured concentrations of pectin in milk replacer rather than target concentrations. At the new maximum proposed use level of 0.2%, the estimated exposure of infants 0–12 weeks of age would be up to 360 and 440 mg/kg bw per day at mean and high consumption. The margins of exposure for average and high consumers are 2.9 and 2.4, respectively, when compared with the NOAEL of 1049 mg/kg bw per day. On the basis of a number of considerations, **the Committee concluded that the margins of exposure calculated for the use of pectin at 0.2% in infant formula indicate low risk for the health of infants and are not of concern.**

(continued)

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions
Quinoline Yellow	R[h]	The Committee concluded that it was reasonable to use toxicology data on D&C Yellow No. 10 to support the database for Quinoline Yellow. **The Committee established an ADI of 0–3 mg/kg bw** (rounded value) for Quinoline Yellow on the basis of a NOAEL of 250 mg/kg bw per day for effects on body weight and organ weights in two long-term studies in rats on D&C Yellow No. 10. An uncertainty factor of 100 was applied to account for interspecies and intraspecies variability. The Committee concluded that dietary exposure to Quinoline Yellow for children and all other age groups does not present a health concern.
Rosemary extract	T[i]	**The Committee established a temporary ADI of 0–0.3 mg/kg bw for rosemary extract, expressed as carnosic acid and carnosol,** on the basis of a NOAEL of 64 mg carnosic acid + carnosol/kg bw per day, the highest dose tested in a short-term toxicity study in rats, with application of a 200-fold uncertainty factor. This uncertainty factor incorporates a factor of 2 to account for the temporary designation of the ADI. The Committee made the ADI temporary pending the submission of studies to elucidate the potential developmental and reproductive toxicity of the rosemary extract under consideration. An additional uncertainty factor to account for the lack of a chronic toxicity study was not considered necessary based on the absence of adverse effects in the short-term toxicity studies at doses up to and including the highest dose tested. **The temporary ADI applies to rosemary extract that meets the specifications prepared at the present meeting. It will be withdrawn if the required data are not provided by the end of 2018.** The Committee noted that the dietary exposure estimates for rosemary extract for high consumers, 0.09–0.81 mg/kg bw per day (as carnosic acid plus carnosol), may exceed the upper bound of the temporary ADI by up to 2.7-fold (for young children at the top end of the range of estimated dietary exposures). Based on the conservative nature of the dietary exposure assessments, in which it was assumed that all foods contained rosemary extracts at the maximum use level, the Committee concluded that this exceedance of the temporary ADI does not necessarily represent a safety concern.
Steviol glycosides	N[j] N,T[k]	**The Committee confirmed the ADI of 0–4 mg/kg bw, expressed as steviol. The Committee also confirmed that rebaudioside A from multiple gene donors expressed in *Yarrowia lipolytica* is included in the ADI.** The Committee concluded that it was not necessary to make the ADI temporary because the requested information to complete the specifications refers only to an update of the method and has no safety implication. The Committee noted that the predicted maximum dietary exposure to steviol glycosides of 4.0–4.4 mg/kg bw per day for young children who were high consumers exceeded the upper bound of the ADI (up to 110%), but the ADI was not exceeded for other age groups. Considering the conservative nature of the dietary exposure estimate, based on maximum use levels applied to all food consumed from categories with permissions for use in the countries assessed, steviol glycosides are not likely to present a health concern for any age group.

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions
Tartrazine	R[l]	**The Committee established an ADI of 0–10 mg/kg bw**, on the basis of a NOAEL of 984 mg/kg bw per day for reductions in body weight in a chronic rat study, with application of a 100-fold uncertainty factor to account for interspecies and intraspecies variability. **The Committee withdrew the previous ADI of 0–7.5 mg/kg bw per day.** The Committee noted that the dietary exposure estimate for children aged 1–10 years was below the upper bound of the ADI and concluded that dietary exposure to tartrazine for the general population, including children, does not present a health concern.
Xanthan gum	R[m]	A NOAEL of 750 mg/kg bw per day was established for xanthan gum in neonatal pigs, which are an appropriate animal model for the assessment of the safety of the additive for infants. The margin of exposure based on this NOAEL and the conservative estimate of xanthan gum intake of 220 mg/kg bw per day by infants (high energy requirements for fully formula-fed infants) is 3.4. On the basis of a number of considerations, **the Committee concluded that the consumption of xanthan gum in infant formula or formula for special medical purposes intended for infants is of no safety concern at the maximum proposed use level of 1000 mg/L.**

N: new specifications; R: existing specifications revised; T: tentative specifications

[a] The method for the determination of lead was changed from atomic absorption to any method appropriate to the specified level. Updated HPLC conditions were added for determining subsidiary colouring matters and organic compounds other than colouring matters. The method of assay was changed to visible spectrophotometry, and spectrophotometric data were provided for the colour dissolved in water.

[b] For carob bean gum and carob bean gum (clarified). A limit for lead of 0.5 mg/kg for use in infant formula was introduced. There were insufficient data to set a limit for arsenic. The method descriptions for the determination of lead and sample preparation for residual solvents were updated.

[c] The Committee noted that the current use level of carob bean gum for infant formula or for formula for special medical purposes intended for infants in CODEX STAN 72-1981 (1000 mg/L) is much lower than the proposed use level (10 000 mg/L).

[d] The tentative status was removed. The assay value was increased from 60% to 75% for total carotenoids, a method for the determination of the proportion of zeaxanthin in total carotenoids (<10%) was included and amendments were made to the method for the determination of waxes.

[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 tentative status was removed.

[g] The limit for lead for general use was lowered from 5 to 2 mg/kg, a limit for lead of 0.5 mg/kg for use in infant formula was introduced and the method descriptions for the determination of lead and sample preparation for residual solvents were updated.

[h] The tentative status was removed. Methods for determining lead and zinc were revised, the titanium trichloride assay was replaced with assay by spectrophotometry, the maximum wavelength of absorbance and absorptivity value for the colour dissolved in water were added, and HPLC conditions for determining the subsidiary colouring matters and organic compounds other than colouring matters and for assaying the colouring components were added.

[i] The published gas chromatography–mass spectrometry method for the determination of key volatiles of rosemary extract was included. Additional information is required to finalize the specifications (see section 5).

[j] A new specifications monograph (Rebaudioside A from Multiple Gene Donors Expressed in *Yarrowia lipolytica*) was prepared for the yeast-derived product.

[k] New tentative specifications for steviol glycosides were established, including a new title name (Steviol Glycosides from *Stevia rebaudiana* Bertoni) to reflect the separation of specifications by source material. The Definition and Assay specification was expanded from nine named leaf-derived steviol glycosides to include any mixture of steviol glycoside compounds derived from *Stevia rebaudiana* Bertoni, provided that the total percentage of steviol glycosides is not less than 95%. Additional information is required to finalize the specifications (see section 5).

[l] The method for the determination of lead was changed from atomic absorption to any method appropriate to the specified level. Updated HPLC conditions were added for determining subsidiary colouring matters and organic compounds other than colouring matters. The method of assay was changed to visible spectrophotometry, and spectrophotometric data were provided for the colour dissolved in water.

[m] The limit for lead in xanthan gum was maintained at 2 mg/kg for general use, and a limit for lead of 0.5 mg/kg for use in infant formula was introduced. The test method for the determination of residual solvents that employs a gas chromatographic method using a packed column was replaced with a method using a capillary column.

Food additives considered for specifications only

Food additive	Specifications
Acetylated distarch adipate	R, T[a,b]
Acetylated distarch phosphate	R, T[a,b]
Acetylated oxidized starch	R[b]
Acid treated starch	R, T[a,b]
Alkaline treated starch	R, T[a,b]
Aspartame	R[c]
Bleached starch	R, T[a,b]
Cassia gum	R, T[d]
Citric and fatty acid esters of glycerol	R[e]
Dextrin roasted starch	R, T[a,b]
Distarch phosphate	R, T[a,b]
Enzyme-treated starch	R, T[a,b]
Hydroxypropyl distarch phosphate	R, T[a,b]
Hydroxypropyl starch	R, T[a,b]
Monostarch phosphate	R, T[a,b]
Octanoic acid	R[f]
Oxidized starch	R, T[b]
Phosphated distarch phosphate	R, T[a,b]
Starch acetate	R[b]
Starch sodium octenyl succinate	R, T[a,b,g]
Total colouring matters	R[h]

R: existing specifications revised; T: tentative specifications

[a] Additional information is required for the removal of the tentative status (see section 5).

[b] The Committee noted that all the modified starches may additionally be subjected to bleaching and therefore included the appropriate purity tests in the revised specifications.

[c] The purity tests for 5-benzyl-3,6-dioxo-2-piperazineacetic acid and other optical isomers were replaced by new published and validated high-performance liquid chromatography (HPLC) tests. The identification characteristic for solubility in ethanol was changed from "slightly soluble" to "practically insoluble or insoluble".

[d] The Committee decided to remove the current method for anthraquinones from the specifications and make the specifications tentative. The additional information required for the removal of the tentative status is noted under section 5.

[e] A limit for lead of 0.5 mg/kg for use in infant formula was introduced.

[f] The infrared spectrum identity test conditions and the reference spectrum were included.

[g] The limit for lead (2 mg/kg) was maintained, as no data were received in response to the call for data.

[h] Procedure 1 (water-soluble colouring matters) and Procedure 3 (lakes) were revised. Table 1 was revised to give spectrophotometric data for 17 synthetic colours, their aluminium lakes, cochineal extract and carmine dissolved in water and buffers. Reagents, solution preparations and sample preparation information were added. Equations shown in Procedures 1, 2 and 3 were edited. The tentative status of the method was removed. Where available, information on the wavelength of maximum absorbance, absorptivity and/or specific absorbance (including information on the solvent used) for the 17 synthetic colours and cochineal extract used to form a lake was included in Table 1 of the revised method. The Committee noted that chloroform is listed as a reagent in Procedure 2 (organic solvent–soluble colouring matters) and decided that efforts should be made to replace it.

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

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

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class III			
2-(2-Hydroxy-4-methyl-3-cyclohexenyl)-propionic acid gamma-lactone	2223	N	No safety concern
2-(2-Hydroxyphenyl)-cyclopropanecarboxylic acid delta-lactone	2224	N	No safety concern

N: new specifications

B. Aliphatic and aromatic amines and amides

The Committee concluded that the concerns previously expressed by the Committee at its sixty-ninth meeting as to in vivo genotoxicity and how to address the kidney effects and identify a NOAEL have not been sufficiently addressed and that the Procedure still could not be applied to 2-isopropyl-*N*,2,3-trimethylbutyramide (No. 1595).[8]

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class III			
N1-(2,3-Dimethoxybenzyl)-*N2*-(2-(pyridin-2-yl)ethyl)oxalamide	2225	N	No safety concern
(*R*)-*N*-(1-Methoxy-4-methylpentan-2-yl)-3,4-dimethylbenzamide	2226	N	No safety concern
(*E*)-*N*-[2-(1,3-Benzodioxol-5-yl)ethyl]-3-(3,4-dimethoxyphenyl)prop-2-enamide	2227	N	No safety concern
(*E*)-3-Benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide	2228	N	No safety concern
N-Ethyl-5-methyl-2-(methylethenyl)-cyclohexanecarboxamide	2229	N[a]	Additional data required to complete evaluation
N-Ethyl-2,2-diisopropylbutanamide	2005	M[b]	Additional data required to complete evaluation
N-(2-Hydroxyethyl)-2,3-dimethyl-2-isopropylbutanamide	2010	M[b]	Additional data required to complete evaluation
N-(1,1-Dimethyl-2-hydroxyethyl)-2,2-diethylbutanamide	2011	M[b]	Additional data required to complete evaluation

M: existing specifications maintained; N: new specifications

[a] The specifications include a statement that the safety evaluation for the flavouring agent had not been completed.

[b] The statement currently contained in the specifications indicating that the safety evaluation had not been completed will be maintained.

[8] The statement currently contained in the specifications indicating that the safety evaluation had not been completed will be maintained.

C. Aliphatic secondary alcohols, ketones and related esters

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class III			
9-Decen-2-one	2216	N	No safety concern
Yuzunone	2217	N	No safety concern
1,5-Octadien-3-ol	2218	N	No safety concern
3,5-Undecadien-2-one	2219	N	No safety concern
3-Methyl-5-(2,2,3-trimethylcyclopent-3-en-1-yl)pent-4-en-2-ol	2220	N	No safety concern
(±)-1-Cyclohexylethanol	2221	N	No safety concern

N: new specifications

D. Cinnamyl alcohol and related substances

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class I			
Ethyl alpha-acetylcinnamate	2211	N	No safety concern
Ethyl 2-hydroxy-3-phenylpropionate	2213	N	No safety concern
Structural class III			
3-(3,4-Methylenedioxyphenyl)-2-methylpropanal	2212	N[a]	Additional data required to complete evaluation
Cinnamaldehyde propyleneglycol acetal	2214	N	No safety concern
2-Phenylpropanal propyleneglycol acetal	2215	N	No safety concern

N: new specifications
[a] The specifications include a statement that the safety evaluation for the flavouring agent had not been completed.

E. Tetrahydrofuran and furanone derivatives

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class II			
2,5-Dimethyl-3(*2H*)-furanone	2230	N	No safety concern
Structural class III			
2,5-Dimethyl-4-ethoxy-3(*2H*)-furanone	2231	N	No safety concern
5-Methyl-3(*2H*)-furanone	2232	N	No safety concern
Ethyl 2,5-dimethyl-3-oxo-4(*2H*)-furyl carbonate	2233	N	No safety concern
4-Acetyl-2,5-dimethyl-3(*2H*)-furanone	2234	N[a]	Additional data required to complete evaluation

N: new specifications
[a] The specifications include a statement that the safety evaluation for the flavouring agent had not been completed.

Flavouring agents considered for specifications only

Flavouring agent	No.	Specifications
3-Methyl-2-(2-pentenyl)-2-cyclopenten-1-one	1114	R[a]
6,10-Dimethyl-5,9-undecadien-2-one	1122	R[b]
3-Ammonium isovalerate	1203	R[c]
Theaspirane	1238	R[d]
alpha-Bisabolol	2031	R[e]
Glutamyl-valyl-glycine	2123	R[f]

[a] The Committee changed the assay minimum from greater than 98% as the *cis* isomer to greater than 95% as a sum of isomers, revised the ranges for refractive index and specific gravity, and introduced new information on the isomeric composition of the flavouring agent.

[b] The Committee indicated that the assay minimum was for a sum of isomers, changed the Chemical Abstracts Service (CAS) number, revised the information for solubility in ethanol, revised the ranges for refractive index and specific gravity, and introduced new information on the isomeric composition of the flavouring agent.

[c] The Committee corrected the molecular weight and chemical formula and revised the melting point range for the flavouring agent.

[d] The Committee lowered the assay minimum from greater than 97% (sum of stereoisomers) to greater than 85% (sum of stereoisomers), revised the ranges for refractive index and specific gravity, and introduced new information on the isomeric composition and secondary components of the flavouring agent.

[e] The Committee changed the assay minimum from greater than 93% to greater than 95% as a sum of isomers, added a second CAS number, revised the ranges for refractive index and specific gravity, clarified the range of the secondary component, and introduced new information on the isomeric composition of the flavouring agent.

[f] The Committee lowered the assay minimum from greater than 99% to greater than 95%.

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
Cinnamyl alcohol and related substances				
2214	Cinnamaldehyde propyleneglycol acetal	92%	Cinnamaldehyde (No. 656) (4–5%)	Cinnamaldehyde (No. 656) has previously been evaluated by the Committee to be of no safety concern at estimated dietary exposures when used as a flavouring agent.
Aliphatic secondary alcohols, ketones and related esters				
2220	3-Methyl-5-(2,2,3-trimethylcyclopent-3-en-1-yl)pent-4-en-2-ol	90%	6-(2,2,3-trimethylcyclopent-3-en-1-yl)-hex-5-en-3-ol (CAS No. 68480-05-7), present at 4–5%, and 3-methyl-5-(2,2,3-trimethylcyclopent-3-en-1-yl)pent-3-en-2-one (CAS No. 65113-95-3) (1–2%)	These substances are structurally similar to No. 2220 and are considered not to present a safety concern at estimated dietary exposures from use of No. 2220 as a flavouring agent.
Alicyclic, alicyclic-fused and aromatic-fused ring lactones				
2224	2-(2-hydroxyphenyl)-cyclopropanecarboxylic acid delta-lactone	93%	Dihydrocoumarin (No. 1171) (2–3%)	Dihydrocoumarin (No. 1171) has previously been evaluated by the Committee to be of no safety concern at estimated dietary exposures when used as a flavouring agent.
Tetrahydrofuran and furanone derivatives				
2233	Ethyl 2,5-dimethyl-3-oxo-4(*2H*)-furyl carbonate	90%	2,5-Dimethylfuran-3,4-diyl diethyl bis(carbonate) (5–6%)	2,5-Dimethylfuran-3,4-diyl diethyl bis(carbonate) is predicted to undergo rapid hydrolysis of the carbonate moieties to form the unstable intermediate of 2,5-dimethylfuran-3,4-diol, which will rapidly oxidize under acidic conditions to form 2,5-dimethyl-4-hydroxy-3(*2H*)-furanone (No. 1446).
Specifications submitted for revision only				
1238	Theaspirane	85%	6-Methylidene-2,10,10-trimethyl-1-oxaspiro[4.5]decane (1%) 4,6,10,10-Tetramethyl-5-oxabicyclo-[4.4.0]dec-1-ene (4–5%) 2-(1,3-Butadienyl)-1,3,3-trimethyl-1-cyclohexene (1–1.5%) 6-(2-Butenylidene)-1,5,5-trimethyl-1-cyclohexene (1.5–2%) 3,4-Dihydro-1,1,6-trimethyl-(*2H*)-naphthalene (~1%)	The Cramer structural classes were determined and the exposure estimated based on the MSDI exposure estimate for theaspirane (No. 1238) determined at the sixty-first JECFA (Annex 1, reference *166*) and the percentage of the secondary component present in the flavouring. All estimated exposures were well below the respective class thresholds. It was therefore concluded that the five secondary components were of no safety concern at current estimated levels of exposure.

Annex 4

Meeting agenda

Food and Agriculture Organization of the United Nations

World Health Organization

82nd JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES (JECFA)
WHO Headquarters, Geneva, 7 – 16 June

Opening:
Room C, 7 June at 9.30h

Draft Agenda

1. Opening

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

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

4. Adoption of Agenda

5. Matters of interest arising from previous Sessions of the Codex Committee on Food Additives (CCFA)

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:
   - *Acacia polyacantha var. Campylacantha*, kakamut gum, arabino-galactan protein complex
   - Allura Red AC (INS 129)
   - Carob bean gum (INS 410)
   - Octenyl succinic acid modified gum arabic (INS 423)
   - Pectin

- Quinoline Yellow
- Rosemary extract (INS 392)
- Steviol glycosides (INS 960)
- Tartrazine (INS 102)
- Xanthan gum (INS 415)

7.2 Food additives for revision of specifications and analytical methods:
- Cassia gum (INS 427)
- Citric and fatty acid esters of glycerol (INS 472c)
- Lutein esters from *Tagetes erecta*
- Modified starches (INS 1400–1405, 1410, 1412–1414, 1420, 1422, 1440, 1442, 1450, 1451)
- Octanoic acid
- Sodium dihydrogen phosphate (INS 339(i))
- Starch sodium octenyl succinate (INS 1450)
- Total Colouring Matters Content (Tentative) (Volume 4)

Flavourings

7.3 Toxicological evaluation, exposure assessment and establishment of specifications for certain flavourings
- Cinnamyl alcohol and related substances
- Aliphatic secondary alcohols, ketones and related esters
- Alicyclic, alicyclic-fused and aromatic-fused ring lactones
- Aliphatic and aromatic amines and amides
- Tetrahydrofuran and furanone derivatives

8. Revision of specifications for certain flavourings

9. Other matters to be considered (general considerations)
   For discussion: Proposal for revised decision tree for the evaluation of flavours

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

11. Adoption of the report.

SELECTED WHO PUBLICATIONS OF RELATED INTEREST

Evaluation of Certain Veterinary Drug Residues in Food
Eighty-first Report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 997, 2016 (110 pages)

Toxicological Evaluation of Certain Veterinary Drug Residues in Food
Eighty-first Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)
WHO Food Additives Series, No. 72, 2016 (162 pages)

Evaluation of Certain Food Additives and Contaminants
Eightieth Report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 995, 2016 (114 pages)

Safety Evaluation of Certain Food Additives and Contaminants
Eightieth Meeting of the Joint FAO/WHO Expert Committee on Food Additives
WHO Food Additives Series, No. 71, 2015 (132 pages)

Evaluation of Certain Food Additives
Seventy-ninth Report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 990, 2015 (124 pages)

Safety Evaluation of Certain Food Additives
Seventy-ninth Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)
WHO Food Additives Series, No. 70, 2015 (369 pages)

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)

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, with a view to concluding as to safety concerns and to preparing specifications for identity and purity.

The first part of the report contains a general discussion of the principles governing the toxicological evaluation of and assessment of dietary exposure to food additives, including flavouring agents. A summary follows of the Committee's evaluations of technical, toxicological and dietary exposure data for 10 food additives (Allura Red AC; carob bean gum; lutein esters from *Tagetes erecta*; octenyl succinic acid (OSA)–modified gum arabic; pectin; Quinoline Yellow; rosemary extract; steviol glycosides; tartrazine; and xanthan gum) and five groups of flavouring agents (alicyclic, alicyclic-fused and aromatic-fused ring lactones; aliphatic and aromatic amines and amides; aliphatic secondary alcohols, ketones and related esters; cinnamyl alcohol and related substances; and tetrahydrofuran and furanone derivatives).

Specifications for the following food additives were revised: aspartame; cassia gum; citric and fatty acid esters of glycerol (CITREM); modified starches; octanoic acid; starch sodium octenyl succinate; and total colouring matters.

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

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