

Evaluation of certain food additives

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



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Eighty-seventh meeting of the Joint FAO/WHO Expert Committee on Food Additives

Rome, 4–13 June 2019

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List of abbreviations

ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
AL-HMF	acidified liquid human milk fortification
ALT	alanine aminotransferase
AST	aspartate aminotransferase
ATBC	Alpha-Tocopherol, Beta Carotene Cancer Prevention
AUC _{0-∞}	area under the concentration–time curve from time 0 to infinity
BMD	benchmark dose
bw	body weight
CARET	Beta-Carotene and Retinol Efficacy Trial
CAS	Chemical Abstracts Service
CCFA	Codex Committee on Food Additives
CCFA51	Fifty-first Session of the Codex Committee on Food Additives
CI	confidence interval
CIFOCoss	FAO/WHO Chronic Individual Food Consumption database – summary statistics
CITREM	citric and fatty acid esters of glycerol
C _{max}	maximum concentration
DNA	deoxyribonucleic acid
EFSA	European Food Safety Authority
EHC 240	Environmental Health Criteria, No. 240
F ₁	first filial generation
FAIM	Food Additive Intake Model
FAO	Food and Agriculture Organization of the United Nations
FSMP	formulas for special medical purposes for infants
GEMS/Food	Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme
GIFT	Global Individual Food consumption data Tool
GMP	Good Manufacturing Practice
GSFA	(Codex) General Standard for Food Additives
HMF	human milk fortification
HPLC	high-performance liquid chromatography
hPXR	human pregnane X receptor
INS	International Numbering System for Food Additives
IU	International Units
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
LD ₅₀	median lethal dose
LOAEL	lowest-observed-adverse-effect level



MOE	margin of exposure
mPXR	mouse pregnane X receptor
NAL-HMF	non-acidified liquid human milk fortification
NHANES	National Health and Nutrition Examination Survey (USA)
NOAEL	no-observed-adverse-effect level
OECD	Organisation for Economic Co-operation and Development
PTWI	provisional tolerable weekly intake
RIVM	Dutch National Institute for Public Health and the Environment
rPXR	rat pregnane X receptor
RR	relative risk
T ₃	triiodothyronine
T ₄	thyroxine
T _{max}	time to reach the maximum concentration
TSH	thyroid stimulating hormone
USA	United States of America
WHO	World Health Organization



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

Safety evaluation of certain food additives. WHO Food Additives Series, No. 78, 2019.

Specifications are issued separately by FAO under the title:

Compendium of food additive specifications. FAO JECFA Monographs 23, 2019.



Dedication

Ms Inge Meyland

Institute of Food Safety and Nutrition, Denmark (retired)

It was with great sadness that the Committee noted the passing of Ms Inge Meyland. Inge was an active member of JECFA until 2016 and played a vital role in shaping the approaches to the development of specifications for food additives. She was instrumental in developing the collection of JECFA specifications published in Monograph 1, *Combined Compendium of Food Additive Specifications*, reproducing for the first time all the specifications monographs from the 1st to the 65th meeting (1956–2005) of JECFA. She continued to serve as an expert and chaired many meetings with an unwavering dedication to scientific excellence and collegiality. Inge was a cornerstone of JECFA over many years, and her deep knowledge of the subject matter and long-standing experience made her famous for her “institutional memory”. Her warm personality, bright mind and great sense of humour will always be remembered.

Inge will be thoroughly missed by her peers and friends in the scientific community. In recognition of her service, the Committee dedicates this report to the memory of Ms Inge Meyland.



1. Introduction

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) met in Rome from 4 to 13 June 2019. The meeting was opened on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) by Dr Markus Lipp, Head of Food Safety and Quality, Agriculture and Consumer Protection Department, FAO.

Dr Lipp preceded his opening remarks by welcoming Dr Yongxiang Fan, Chairperson of the Codex Committee on Food Additives (CCFA), and all other meeting participants. Dr Lipp highlighted the roles and responsibilities that JECFA has in the framework of the international food safety standard development work of the Codex Alimentarius Commission. He reminded the JECFA experts about their responsibility to elaborate the most unbiased and best scientific advice possible.

Dr Lipp emphasized that participants had been invited not as representatives of their employer or country, but to serve solely in their capacity as scientific experts to provide sound and independent scientific advice to generate food standards designed to be protective of health for all consumers and trade-inclusive for all regions and countries. He finished by urging the attendees to be as open and transparent as possible and emphasizing that scientific excellence will require the input from all and the courage to ask critical questions.

1.1 Declarations of interests

The Joint Secretariat informed the Committee that all experts participating in the eighty-seventh meeting had completed declaration of interest forms. No conflicts of interest were identified.

1.2 Modification of the agenda

No data were submitted on β -apo-8'-carotenoic acid methyl and ethyl esters, and these were removed from the evaluation of carotenoids (see agenda item 7.1 in [Annex 4](#)). The Committee also renamed the remaining carotenoids on the agenda (β -carotene, β -carotene from *Blakeslea trispora* and β -apo-8'-carotenal) as carotenoids (provitamin A). β -Carotene-rich extract from *Dunaliella salina* was included in the group of carotenoids (provitamin A).

Citric and fatty acid esters of glycerol (CITREM) was added to agenda item 7.3 for revision of specifications.



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 86 previous meetings of the Committee (Annex 1). The present meeting was convened on the basis of a recommendation made at the eighty-sixth meeting (Annex 1, reference 241).

The tasks before the Committee were to:

- elaborate further principles for evaluating the safety of food additives (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 (section 3 and Annex 2).

2.1 Report from the Fifty-first Session of the Codex Committee on Food Additives (CCFA)

Dr Yongxiang Fan, Chairperson of CCFA, supported by the Codex Secretariat, provided the Committee with an update on the work of CCFA since the eighty-sixth meeting of JECFA (Annex 1, reference 241).

The Fifty-first Session of CCFA (CCFA51) noted the conclusions of the eighty-sixth meeting of JECFA on the safety of nine substances and 69 flavourings (2). CCFA51 agreed to include basic methacrylate copolymer (International Numbering System for Food Additives [INS] 1205), lutein from *Tagetes erecta* (INS 161b(i)) and zeaxanthin (synthetic) (INS 161h(i)) in Table 3 (Additives Permitted for Use in Food in General, Unless otherwise Specified, in Accordance with GMP [Good Manufacturing Practice]) of the Codex *General Standard for Food Additives* (GSFA) (CODEX STAN 192-1995) (3). CCFA51 solicited members to provide more information or data to JECFA to allow the Committee to complete its evaluations of anionic methacrylate copolymer (INS 1207), neutral methacrylate copolymer (INS 1206) and spirulina extract (INS 134) and noted that no action was necessary for other substances.

CCFA51 forwarded specifications for the identity and purity of six food additives (one new specification and five revised specifications) and 27 flavouring agents (20 new specifications and seven revised specifications) prepared by the eighty-sixth meeting of JECFA and recommended them to the Forty-second Session of the Codex Alimentarius Commission for adoption. CCFA51 agreed on a revised priority list of substances for evaluation (or re-evaluation) by JECFA, which included 24 food additives (10 food additives were ranked as the highest

priority), 76 flavouring agents and 29 processing aids. CCFA51 agreed to amend the circular letter on the priority list for the purpose of clarification.

CCFA51 also made recommendations on 155 provisions already in the Codex step procedure and/or already adopted and discussed 102 proposed new and/or revised provisions of the GSFA. CCFA51 made major progress on replacing Note 161 by developing alternative wording for Note 161 relating to the use of sweeteners. CCFA51 agreed to establish both ingoing and residue levels for nitrates and nitrites in the GSFA.

CCFA51 agreed to delete red 2G (INS 128) and distarch glycerol (INS 1411) from the *Class Names and the International Numbering System for Food Additives* (CXG 36-1989) (4). The name of INS 160a(iv) was changed from “Carotenes, beta-, algae” to “ β -carotene-rich extract from *Dunaliella salina*”. CCFA51 also completed the work on the alignment of the food additive provisions related to 23 commodity standards (13 standards for milk and milk products, two standards for sugars, two standards for natural mineral waters, three standards for cereals, pulses and legumes, three standards for vegetable proteins).

CCFA51 considered the issue of group food additives. It was understood that JECFA was going to re-evaluate two groups of food additives (carotenoids and 2-phenylphenols or *ortho*-phenylphenols) and had a general discussion on the use of the terms “group ADI [acceptable daily intake]” and “group PTWI [provisional tolerable weekly intake]” as well as how JECFA assigns group ADIs. The outputs of JECFA will guide the future considerations of CCFA in this regard.

The Fifty-second Session of CCFA will continue its routine work, including the development of the GSFA, alignments of food additive provisions in the Codex commodity standards with the corresponding provisions of the GSFA (3) and revisions to the *Class Names and the International Numbering System for Food Additives* (CXG 36-1989) (4).

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 *Principles and methods for the risk assessment of chemicals in food* (Environmental Health Criteria, No. 240 [EHC 240]), published in 2009 (5).

2.2.1 Application of group ADIs

At the Fiftieth Session of CCFA, the Codex Secretariat noted that some food additives – such as provitamin A carotenoids (i.e. synthetic β -carotenes, β -carotene from *Blakeslea trispora*, β -apo-8'-carotenal and methyl and ethyl esters of β -apo-8'-carotenoic acid); chlorophylls and chlorophyllins, copper complexes;

and polyoxyethylene sorbitan esters (i.e. polyoxyethylene (20) sorbitan esters of lauric, stearic, palmitic and oleic acids and triesters of stearic acid) – were listed under the same food additive heading in the GSFA, despite not being included in a group ADI. The Codex Secretariat sought clarification from the present Committee on the application of group ADIs.

In making recommendations on the safety of food additives, the Committee takes into consideration the principles regarding group ADIs contained in EHC 240 (5).

The Committee noted that most of the food additives about which CCFA had sought advice had been last considered as groups at several meetings up to and including the twenty-third meeting in 1980 and that the Committee did not explicitly use the term group ADI at those early meetings. Of these food additives, the Committee was able to confirm that group ADIs should have been established for the chlorophylls and chlorophyllins (copper complexes), polyoxyethylene sorbitan esters (polysorbates), ascorbyl esters, ethylenediaminetetraacetates, thiodipropionates, ferrocyanides, tartrates, stearyl lactylates and iron oxide food additives.

For nitrates and nitrites, the respective ADIs are expressed as the ions and therefore encompass the different salts. The group ADI for steviol glycosides, expressed as steviol, includes the whole family of steviol glycosides. The Committee was also able to confirm that the PTWI of 2 mg/kg body weight (bw) for aluminium and its salts, when expressed as aluminium, refers to all aluminium salts used in food additives, as well as other sources of aluminium.

An “unconditional” ADI of 0–0.2 mg/kg bw for 2-phenylphenol was first established by JECFA at its eighth meeting in 1964. According to FAO documents, 2-phenylphenol and sodium *o*-phenylphenate were first evaluated by the 1962 JECFA for their use as a post-harvest treatment of fruits and vegetables to protect against microbial damage during storage and distribution. The current FAO specifications still refer to this use. In 1999, the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) established an ADI of 0–0.4 mg/kg bw for 2-phenylphenol; an ADI was not established for the sodium salt because it rapidly dissociates to 2-phenylphenol (6). 2-Phenylphenol has a minor use as a flavouring agent, and, during its evaluation at the fifty-fifth meeting of JECFA, the Committee cited the most recent ADI established by JMPR for its risk assessment ([Annex 1](#), reference 149). In view of its major use as a post-harvest treatment of fruits and vegetables, the Committee is seeking advice from Codex on its current usage as a food additive.

The Committee noted that provitamin A carotenoids were evaluated at the current meeting (see [section 3.1.3](#)).

2.2.2 Clarification of ADI “not specified”

Codex requested clarification of the use of the term “ADI ‘not specified’” by JECFA, particularly with respect to addition of food additives to Table 3 of the GSA (Additives Permitted for Use in Food in General, Unless otherwise Specified, in Accordance with GMP).

The Committee confirmed its definition of “ADI ‘not specified’” (5):

A term applicable to a food substance of very low toxicity that, on the basis of the available chemical, biochemical and toxicological data as well as the total dietary intake of the substance (from its use at the levels necessary to achieve the desired effect and from its acceptable background in food), does not, in the opinion of the Joint FAO/WHO Expert Committee on Food Additives, represent a hazard to health. For that reason, and for reasons stated in 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: that is, it should be technologically efficacious and should be used at the lowest level necessary to achieve this effect, it should not conceal inferior food quality or adulteration, and it should not create a nutritional imbalance.

Thus, the definition is based upon information on both toxicity and dietary exposure (intake). A conclusion that a substance is of very low toxicity could be based, for example, upon evidence that the substance did not show adverse effects at the highest doses tested in relevant toxicological studies, is poorly absorbed and does not bioaccumulate, and does not contain toxicologically relevant impurities. The estimate of total dietary exposure (intake) is based upon the uses proposed at the time of the evaluation.

The Committee noted that Guideline 2 (Food Additives with an ADI of “Not Specified”) of the GSA (CODEX STAN 192-1995) (3) specifies:

When an additive has been allocated an ADI “not specified” it could in principle, be allowed for use in foods in general with no limitation other than in accordance with Good Manufacturing Practices (GMP). It should, however, be born [sic] in mind that ADI not specified does not mean that unlimited intake is acceptable. The term is used by JECFA in case [sic] where “on the basis of the available data (chemical, biochemical, toxicological, and other) the total daily intake of the substance arising from its use at the levels necessary to achieve the desired effect and from its acceptable background in food does not, in the opinion of the Committee, represent a hazard to health”. If, therefore, a substance is used in larger amounts and/or in a wider range of foods than originally envisaged by JECFA it may be necessary to consult JECFA to ensure that the new uses fall within the evaluation. For example a substance may have been evaluated as a humectant

without including a later use as a bulk sweetener, which could give considerable [sic] higher intake.

The Committee endorses Guideline 2 of the GSFA and recommends that it be applied by addition of appropriate qualifications in Table 3 of the GSFA.

2.2.3 Update of guidance on evaluation of enzyme preparations (EHC 240)

The Committee was informed about activities of an expert working group established in 2018 to discuss available information on the safety of enzymes used in food and current practices of the food enzyme industry. This activity is being undertaken within the context of a joint FAO/WHO project to update various chapters of *Principles and methods for the risk assessment of chemicals in food* (EHC 240) (5).

The starting point of the discussion was a background document prepared from a review of the current literature and conversations with representatives of the food enzyme industry and their technical experts.

It was noted that the current JECFA guidance on the evaluation of enzyme preparations was designed to address the potential toxicity of secondary metabolites generated by some enzyme sources (e.g. *Aspergillus* species) under certain growth conditions. The guidance includes a requirement to conduct genotoxicity tests as well as 90-day oral toxicity tests in animals.

After nearly 15 years of using this guidance to assess the safety of enzyme preparations, JECFA has not identified any that were toxic. The expert working group proposed that the safety of enzyme preparations could be assessed with methodologies using fewer animals (e.g. metabolic profiling of microbial fermentation products, genomic DNA sequencing identifying mycotoxin synthesis genes). The expert working group focused on enzymes from genetically modified microorganisms and the information requirements for their safety evaluation.

The expert working group will propose changes to the relevant sections of EHC 240 and produce a checklist of information required in enzyme submissions for future JECFA evaluations.

The Committee urges the expert working group to finalize its work and make the output available for public comment in time for the JECFA meeting in 2020.

2.2.4 Update of guidance on evaluation of genotoxicity of chemical substances in food (section 4.5 of EHC 240)

The Committee was informed about activities of an FAO/WHO expert working group established in 2018 to update and extend the guidance on evaluation of genotoxicity of chemical substances in food. This activity is being undertaken

within the context of a joint FAO/WHO project to update various chapters of *Principles and methods for the risk assessment of chemicals in food* (EHC 240) (5). The aim of the expert working group is to provide guidance on interpretation of test results, in addition to general descriptions of genotoxicity tests, special considerations for data-poor substances, and considerations for chemically related substances and mixtures. The expert working group will also address recent developments and future directions.

This work is ongoing. A public consultation is intended before finalization.

2.2.5 Update of guidance on dose–response assessment and derivation of health-based guidance values (Chapter 5 of EHC 240)

At the eighty-third meeting of the Committee (in 2016), some general considerations regarding dose–response modelling were discussed. The Committee recommended that an expert working group be established to develop detailed guidance for the application of the methods most suitable to its work, in particular for the use of the benchmark dose (BMD) approach ([Annex 1](#), reference 233). The Committee asked that the expert working group address several aspects, including the use of constraints when fitting models, the use of model averaging, the use of non-parametric methods as alternatives for dose–response risk assessment, the use of biological information for selection of models and transparent presentation of modelling outcomes in JECFA publications.

The Committee was informed that the recommended expert working group was established in 2017 to update and extend the guidance on dose–response assessment and derivation of health-based guidance values. This activity is being undertaken within the context of a joint FAO/WHO project to update various chapters of *Principles and methods for the risk assessment of chemicals in food* (EHC 240) (5).

The work was undertaken electronically and culminated in a meeting of experts in March 2019 in Geneva to revise and update Chapter 5 of EHC 240, including the preparation of more detailed advice on the BMD approach. The draft revised chapter will include guidance on the use of the freely available BMD software (both the United States Environmental Protection Agency Benchmark Dose Software suite of models and PROAST, which was developed by the Dutch National Institute for Public Health and the Environment [RIVM], now available through the European Food Safety Authority [EFSA] as a web tool). The draft guidance will encourage the use of the BMD approach wherever possible and appropriate, but will acknowledge that in some situations, use of the no-observed-adverse-effect level (NOAEL)/lowest-observed-adverse-effect level (LOAEL) approach may still be appropriate. The draft guidance will include a decision-tree to aid decision-making about which approach should be followed.

It is anticipated that a revised draft of Chapter 5 of EHC 240 will be ready in June 2019, to be reviewed by the expert working group. The draft will then go out for public consultation, will be revised if necessary and will be published online as a standalone chapter.

2.2.6 Update of guidance on assessing dietary exposure to chemical substances in food (Chapter 6 of EHC 240)

The Committee was informed about activities of an FAO/WHO expert working group established in 2018 to update and extend the guidance on assessing dietary exposure to chemical substances in food. This activity is being undertaken within the context of a joint FAO/WHO project to update various chapters of *Principles and methods for the risk assessment of chemicals in food* (EHC 240) (5).

A revision of the chapter was required to incorporate technological and methodological changes in dietary exposure assessment, including progress in the use of exposure models and more recently available data and databases.

WHO undertook an initial scoping exercise that identified areas of the current chapter that needed to be reviewed and new areas of work to be included and prepared a first draft of an updated chapter. The draft chapter will be reviewed by a number of dietary exposure experts at a consultation in September 2019. A final draft will be prepared and then released for public comment.

2.2.7 Dietary exposure assessment reporting

In 1996, WHO held an expert consultation that introduced dietary exposure assessment in JECFA's risk assessments for food additives and contaminants. At a 2005 expert consultation to prepare a dietary exposure assessment chapter for what would become *Principles and methods for the risk assessment of chemicals in food* (EHC 240) (5), a tiered process for systematically preparing dietary exposure assessments was elucidated. This process includes 1) a budget or other screening method, 2) international and national dietary exposure assessments based on summary food consumption data (e.g. Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme [GEMS/Food] cluster diets, FAO/WHO Chronic Individual Food Consumption database – summary statistics [CIFOCoss], national/regional surveys, published exposure assessments) and 3) refined dietary exposure assessment using food consumption data derived from individual consumers. In this last step, deterministic and probabilistic assessments could be completed as needed and appropriate. Guidance to JECFA monographers was prepared from these consultations.

At the current meeting, the Committee determined that not all steps of the tiered approach are needed in every case to complete the Committee's evaluations. When preparing monographs, JECFA experts comment on each of the steps as appropriate, but in the report of the meeting, only those assessments

where sufficient data were available to produce reliable estimates of dietary exposure are described and used in the safety assessment. The Committee noted that lack of discussion of any of the steps in report items does not reflect a lack of consideration during the overall evaluation.

2.2.8 Framework for developing specifications for steviol glycosides by method of production

Steviol glycosides are constituents of the leaves of the plant *Stevia rebaudiana* Bertoni and have a sweet taste. The functional use of steviol glycosides in food is as a sweetener. They are approximately 100–300 times sweeter than sucrose.

The major glycosides present in the extract of the leaves from the *Stevia rebaudiana* Bertoni plant are stevioside and rebaudioside A. The minor glycosides include rebaudioside M and rebaudioside D and about 40 other steviol glycosides that have been identified to date. Several minor glycosides have more favourable sensory characteristics than the major glycosides, prompting development of technologies that enhance the proportion of minor glycosides to modify the sensory profile of the articles of commerce. These technologies include the following:

- a. Extraction: a process of hot water extraction from the leaves of *Stevia rebaudiana* Bertoni.
- b. Fermentation: a process in which a genetically modified microorganism is used to produce specific steviol glycosides.
- c. Enzymatic modification: a process in which steviol glycosides that have been extracted from the leaves of *Stevia rebaudiana* Bertoni undergo enzymatic conversion of major steviol glycosides to minor ones.
- d. Enzymatic glucosylation: a process in which steviol glycosides that have been extracted from the leaves of *Stevia rebaudiana* Bertoni undergo enzyme-catalysed reactions to add glucose units to the steviol glycosides via α -(1-4) linkages.

The microorganisms used in the fermentation or in the production of enzymes used to modify steviol glycosides are of safe lineage. The inserted genes are isolated from non-toxic and non-pathogenic sources. Residues from manufacturing processes do not pose any concerns with respect to toxicity or allergenicity.

Steviol glycosides consist of a mixture of compounds containing a steviol backbone conjugated to any number or combination of the principal sugar moieties (e.g. glucose, rhamnose, xylose, fructose, arabinose, galactose, deoxyglucose). Existing specifications for steviol glycosides require that the product consists of $\geq 95\%$ steviol glycosides on the dried basis.

At the present meeting, the Committee reviewed data on the methods of manufacture, identity and purity of steviol glycosides. The Committee noted that the reviewed products consist of $\geq 95\%$ steviol glycosides on the dried basis; the remaining 5% or less consists of residues of starting material and food-grade processing aids, depending on the method of production.

A framework was adopted for developing specifications for steviol glycosides by four different methods of production. Specifications for steviol glycosides produced by different production methods were included as annexes, as below:

- Annex 1: Steviol Glycosides from *Stevia rebaudiana* Bertoni (revised from the specifications monograph for Steviol glycosides from *Stevia rebaudiana* Bertoni [INS 960a] prepared at the eighty-fourth meeting of JECFA [Annex 1, reference 236]).
- Annex 2: Steviol Glycosides from Fermentation (specifications for Rebaudioside A from multiple gene donors expressed in *Yarrowia lipolytica* [INS 960b(i)] prepared at the eighty-second meeting of JECFA [Annex 1, reference 231] were revised to include other steviol glycosides from *Saccharomyces cerevisiae* and *Yarrowia lipolytica*).
- Annex 3: Enzyme Modified Steviol Glycosides (new specifications).
- Annex 4: Enzyme Modified Glucosylated Steviol Glycosides (new specifications, tentative pending further information concerning the analytical methods).

At the present meeting, the Committee determined that no safety issues exist for steviol glycosides produced by any one of these methods resulting in products with $\geq 95\%$ steviol glycosides as per existing specifications. The Committee indicated that the ADI of 0–4 mg/kg bw established at the sixty-ninth meeting of JECFA for steviol glycosides (expressed as steviol) (Annex 1, reference 190) applies to steviol glycosides produced by the four methods indicated in the annexes of the specifications monograph produced at the current meeting.

The Committee recognized that steviol glycosides could be produced via a new method or the modification or combination of the methods currently described in the annexes of the specifications monograph. If the final product meets the current specification of $\geq 95\%$ steviol glycosides, the Committee will evaluate possible impurities from the method of manufacture. When appropriate, the modifications will be introduced into the relevant annex; alternatively, a new annex would be added.

2.3 Food additive specifications and analytical methods

2.3.1 Unsulfonated primary aromatic amines in food colours

At the present meeting, the Committee noted that the analytical method for determining unsulfonated primary aromatic amines in certain synthetic food colours (i.e. Allura Red AC, Amaranth, Azorubine, Brilliant Black PN, Brilliant Blue FCF, Brown HT, Fast Green FCF, Fast Red E, Green S, Indigotine, Lithol Rubine BK, Patent Blue V, Ponceau 4R, Quinoline Yellow, Sunset Yellow FCF and Tartrazine) described in Volume 4 of the *Combined compendium of food additive specifications* (Annex 1, reference 180) is not sufficiently sensitive for determining the impurities at low levels (milligrams per kilogram or below). The Committee also noted that the specification for unsulfonated primary aromatic amines (not more than 0.01%, calculated as aniline) is approximately 100 times higher than equivalent specifications for food colours established by other regulatory authorities. The Committee also noted that more sensitive analytical methods, capable of determining unsulfonated primary aromatic amines at levels of less than 1 mg/kg, had been developed since the publication of Volume 4.

The Committee requests analytical data on unsulfonated primary aromatic amines in the above food colours, along with the analytical methods used, in order to update specifications.

2.3.2 Analytical method for the determination of anthraquinones in cassia gum

At its eighty-second meeting, the Committee made the specifications for cassia gum tentative and requested information on the analytical method for the determination of anthraquinones, including the efficiency of extraction steps and recovery of analytes (Annex 1, reference 230). At the eighty-sixth meeting, the Committee evaluated the high-performance liquid chromatography (HPLC) method submitted, updated the specifications by including the method received, and removed the tentative status for the specifications of cassia gum (Annex 1, reference 241). Based on comments received about the method performance, the Committee, at its current meeting, reviewed the method again and noted that additional investigations were required. Therefore, the Committee decided to make the specifications tentative until a suitable analytical method has been identified.

2.3.3 Update on the review of analytical methods for food additives

The Committee was informed of the ongoing FAO initiative to review analytical methods for food additives. The review was initiated to ensure that the analytical methods referenced in the specifications monographs for food additives are fit-for-purpose and up-to-date. *Combined compendium of food additive specifications, Volume 4, Analytical methods, test procedures and laboratory*

solutions used by and referenced in the food additive specifications (FAO JECFA Monographs 1) was published in 2006. Subsequently, several analytical methods associated with the specifications monographs were either included in individual monographs or published separately. The Committee, at previous meetings, noted that advancements in instrumentation technologies since the publication of Volume 4 necessitate a review of analytical methods in individual specifications monographs as well as in Volume 4.

In total, 470 specifications monographs (excluding enzymes) were reviewed, together with different subsections of Volume 4. The initial findings were as follows:

General:

- Approximately 170 specifications monographs are more than 30 years old. Approximately 70 out of 170 specifications monographs were developed between the third and twentieth meetings of JECFA and contain some outdated methods.
- Three specifications monographs include the functional use of fungicidal agents; these products are unlikely to be used as food additives.
- Some functional uses detailed in monographs are not consistent with the functional classes listed in the INS (e.g. yeast food).

Analytical methods:

- Approximately 30 monographs still use obsolete packed column gas chromatographic methods.
- Some analytical techniques (e.g. titrimetric, spectrophotometric, thin-layer chromatographic/paper chromatographic identification techniques) are still in use, although they may no longer be fit-for-purpose and have been superseded by newer approaches.
- Certain limit tests (e.g. nickel, fluoride, iron) still exist, although quantitative analytical methods are available.
- Volume 4 requires considerable updating and inclusion of sophisticated analytical methods and confirmatory methods, such as liquid chromatography with tandem mass spectrometry, inductively coupled plasma mass spectrometry, X-ray fluorescence, etc.
- Many standard and test solutions given in Volume 4 are currently not in use and need thorough revision.
- Potential compatibility issues for monographs were found in some updates for Volume 4 (e.g. replacing packed column gas

chromatographic methods, use of chloroform, replacing methods for subsidiary dyes and organic compounds other than colouring matters, etc.).

In view of above findings, the Committee recommended that:

- A summary of the major findings be compiled for presentation to CCFA.
- A priority list of updates be constructed based on initial findings, paying particular attention to relevance of the proposed update, impact on Volume 4, potential for creating further disconnects between the monographs and Volume 4, and the number of monographs affected. The list should be presented to JECFA and CCFA at a future meeting.
- An outline of the future activities be created, including:
 - A decision on the future role of Volume 4 and its contents (e.g. reproducing technical background about analytical methods).
 - A mechanism for the separate evaluation of enzyme monographs and connected analytical methods, once the process and requirements for enzyme evaluations are concluded.
 - A decision on presentation of the analytical methods in specifications monographs (e.g. full methods, active links, relevant technical details, a database of methods).
 - A decision on the policy to reference other publications by FAO and other standards development organizations.

2.4 Other matters of interest to the Committee

2.4.1 Update on FAO/WHO Global Individual Food consumption data Tool (GIFT)

The FAO/WHO Global Individual Food consumption data Tool (GIFT) is an open-access online platform, hosted by FAO and supported by WHO, providing access to harmonized individual quantitative food consumption data, especially in low- and middle-income countries. The platform is a growing data repository; in 2018, FAO/WHO GIFT received a 4-year grant from the Bill & Melinda Gates Foundation to transform the platform into a robust global tool that will contain at least 50 datasets in 2022.

FAO/WHO GIFT provides sex- and age-disaggregated microdata, which are needed in the field of nutrition and dietary exposure. To facilitate the use of these data by policy-makers, ready-to-use food-based indicators are provided under the form of infographics for a user-friendly overview of key information by population segments and by food groups. The synergy between the FAO/WHO GIFT platform and the dashboards of FAO/WHO FOSCOLLAB (Global platform

for food safety data and information) hosted by WHO has great potential. In fact, in order to enhance the consistency and reliability of nutrient intake and dietary exposure assessments, all datasets available as microdata in FAO/WHO GIFT are harmonized with the food classification and description system FoodEx2. FoodEx2 is also the system used to map all food chemical occurrence microdata available on FAO/WHO FOSCOLLAB. The combination of the two platforms will make it much easier to perform refined dietary exposure assessments for a large variety of food chemicals in all regions of the world. Moreover, all datasets available as microdata in FAO/WHO GIFT are also being made available as summary statistics on FAO/WHO FOSCOLLAB.

For datasets that are not yet available as microdata in FAO/WHO GIFT, the platform provides an up-to-date inventory of individual quantitative food consumption surveys conducted and ongoing in low- and middle-income countries, with detailed survey information on identified studies.

The FAO/WHO GIFT platform is available at <http://www.fao.org/gift-individual-food-consumption/en/>. The dashboards of FAO/WHO FOSCOLLAB are available at <http://apps.who.int/foscollab>.

2.4.2 Risk assessments of combined dietary exposure to multiple chemicals

The need to integrate exposure to mixtures of chemicals in the risk assessment framework has long been recognized by FAO/WHO. This work is part of a project entitled “EuroMix” funded by the European Commission, under the Horizon 2020 research programme. In this context, WHO and FAO convened an expert consultation² in April 2019 to develop appropriate guidance for risk assessment of combined dietary exposures to multiple chemicals.

The Committee was informed on the key deliberations of the consultation. In particular, it was noted that if a substance under evaluation by JECFA/JMPR has sufficient similarity to an established chemical group previously considered in a risk assessment of combined dietary exposure to multiple chemicals (e.g. organophosphates), the substance should be considered for assessment as part of that group. If a substance under consideration is not part of an established chemical group previously considered, JECFA/JMPR should then determine whether there is a need to include it in a risk assessment of combined dietary exposure to multiple chemicals.

For chemicals that are not part of a previously established group, if the estimated dietary exposure for a single compound under evaluation is more than 10% of the relevant health-based guidance value or the calculated margin of exposure (MOE) is less than 10 times the MOE considered adequate for such a compound for at least one population, the need to include the compound in a

² https://www.who.int/foodsafety/areas_work/chemical-risks/Euromix_Report.pdf?ua=1

risk assessment of combined dietary exposure to multiple chemicals should be considered.

The following questions must be answered to determine which substances should be included: *Is there toxicological evidence for combined effects* (using weight of evidence analysis, expert judgement on structural similarities, toxicological profiles, modes of action, etc.) and *Is there potential for co-exposure (from co-occurrence or internal exposure)* (using trial data, monitoring data, use levels in foods, toxicokinetic data, etc.).

For risk characterization, suitable procedures using dose addition can be applied to identify key risk drivers using either deterministic or probabilistic approaches, including the key chemicals contributing to total dietary exposure and/or foods contributing to exposure from each chemical.

The consultation noted that for DNA-reactive mutagens, special consideration will be needed, and they were not included in the approach proposed by the consultation. Furthermore, synergistic interactions between chemicals may need to be considered separately on a case-by-case basis.

3. Specific food additives (other than flavouring agents)

The Committee evaluated two food additives for the first time and re-evaluated three others. In addition, the Committee evaluated the safety of one previously evaluated food additive for use in formula for special medical purposes intended for infants. Four food additives (including one group of food additives) were considered for revision of specifications only. Information on the safety evaluations and specifications is summarized in [Annex 2](#). Details of further toxicological studies and other information required for certain substances are summarized in [section 5](#).

3.1 Safety evaluations³

3.1.1 Black carrot extract

Explanation

Black carrot extract (INS 163(vi)) is an anthocyanin-containing food colour obtained by acidic aqueous extraction from the root of black, purple or red carrot. The main colouring components are five cyanidin-based anthocyanins.

Black carrot extract has not been evaluated previously by the Committee. The Committee previously evaluated anthocyanins, including the anthocyanin-containing food colour grape skin extract (INS 163(ii)), at its twenty-sixth meeting ([Annex 1](#), reference 59). At that meeting, the Committee established an ADI for anthocyanins in grape skin extract of 0–2.5 mg/kg bw, based on a NOAEL of 225 mg/kg bw per day expressed as anthocyanins from a two-generation reproductive toxicity study in rats [1].

Black carrot extract was placed on the agenda of the present meeting for assessment of its safety, dietary exposure and specifications, at the request of the Fiftieth Session of CCFA [2]. In response to the call for data, a submission was received, which included studies identified from the publicly available literature and information on specifications and dietary exposure. A comprehensive literature search retrieved a number of additional studies, primarily on human pharmacokinetics and absorption, distribution, metabolism and excretion (ADME), and one additional genotoxicity study.

Given the similar aglycone structures of anthocyanins, the large number of studies on anthocyanins from various sources published since the previous assessment of grape skin extract and the lack of toxicity data on black carrot extract itself (only one genotoxicity study was submitted), the Committee decided to review the available data on anthocyanins as a whole. The studies described below therefore include previously evaluated studies on grape skin

³ Numbered references cited in the subsections of section 3.1 are provided at the end of each subsection.

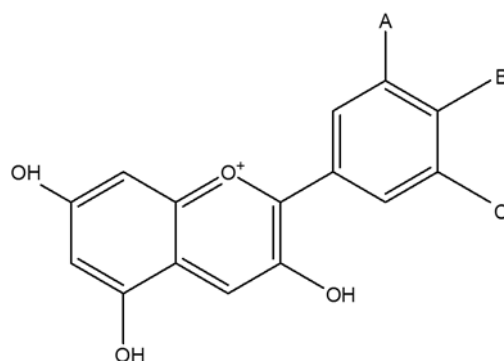
extract (published prior to 1982) as well as new studies on materials containing anthocyanins from a range of sources.

Chemical and technical considerations

Anthocyanins are a large group of related compounds consisting of aglycones such as cyanidin or pelargonidin (Fig. 1) combined with sugars such as galactose or glucose and acylating agents such as caffeic acid or *p*-coumaric acid [3].

Fig. 1

General anthocyanin aglycone structure indicating substitution positions



Aglycone	A	B	C
Cyanidin ^{a,b,c}	-OH	-OH	-H
Pelargonidin ^{a,b}	-H	-OH	-H
Delphinidin ^b	-OH	-OH	-OH
Peonidin ^{a,b}	-O-CH ₃	-OH	-H
Petunidin ^b	-OH	-OH	-O-CH ₃
Malvidin ^{a,b}	-O-CH ₃	-OH	-O-CH ₃

^a Found in black carrot extract.

^b Found in grape skin extract.

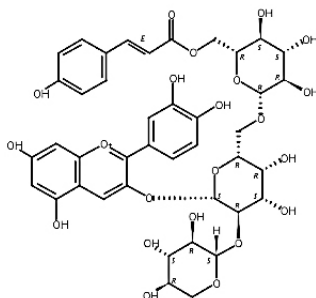
^c The five main anthocyanins in black carrot extract are formed from this aglycone.

Black carrot extract contains five main anthocyanins formed from the aglycone cyanidin substituted at the central hydroxyl position with a sugar moiety consisting of galactose, glucose and/or xylose. Three of the five anthocyanins are acylated with *p*-coumaric, ferulic or sinapinic acid [4]. One of the five main anthocyanins in black carrot extract is shown in Fig. 2. Anthocyanins in black carrot extract are also formed from other aglycones (malvidin, pelargonidin and peonidin; Fig. 1), which are present in minor amounts along with other polyphenols. Other components include proteins, carbohydrates, lipids, fibres,

minerals and water. In contrast to black carrot extract, the predominant aglycone found in anthocyanins in grape skin extract is malvidin [5].

Fig. 2

Cyanidin 3-*p*-coumaroylxylosylglucosylgalactoside, one of the five main anthocyanins in black carrot extract



Black carrot extract is produced by aqueous acidic extraction of the crushed, ground or milled roots of black, purple or red carrot (*Daucus carota* L., ssp. *sativus*) followed by fermentation to decrease sugars. Methanol or ethanol may be produced during the fermentation step. The anthocyanins may be concentrated by ultrafiltration, reverse osmosis or adsorption onto a polymeric resin followed by desorption with ethanol, isopropyl alcohol and/or water. The commercial product may be a liquid or spray-dried powder.

Black carrot extract is intended for use in colouring dairy-based desserts, processed fruit products, processed vegetable products, confectionery, chewing gum, cereals, pastas and noodles, cereal/starch-based desserts, processed rice and soy products, cakes, cookies, pies, preserved egg products, condiments (vinegar, mustard), sauces and gravies, dietetic foods and dietary supplements, non-alcoholic beverages and alcoholic beverages.

Biochemical aspects

The previous Committee, in its evaluation of grape skin extract, concluded that anthocyanins are not absorbed by humans to any great extent (<2%) and pass through the body unchanged ([Annex 1](#), reference 59). More recent studies have shown anthocyanins to be absorbed up to about 12% [e.g. 6–8]; therefore, previously evaluated studies on the ADME of anthocyanins have not been included below.

A number of studies have been carried out in humans to investigate the ADME of anthocyanins. Anthocyanins can be absorbed intact or hydrolysed to the

aglycone and then absorbed. They may also be degraded to phenolic compounds by the gut microbiota before absorption. The primary route of metabolism by the microbiota appears to be cleavage of the heterocyclic flavylum ring followed by dihydroxylation or decarboxylation [9, 10]. The rate and extent of absorption are dependent on the size of the molecule, the type of sugar moiety, the degree of acylation and the matrix in which the anthocyanin mixture is consumed [9]. The gut microbiome is likely to be an important site of metabolism of anthocyanins, and changes in the microbiome may have a significant effect on the metabolic products produced following the consumption of anthocyanins [11].

In recent studies in human volunteers using stable ^{13}C -labelled cyanidin-3-*O*-glucoside, an anthocyanin found in grape skin extract and purple corn colour, bioavailability of about 12% (5% in urine and 7% in breath) was reported. Several metabolites were identified, including carbon dioxide in breath and anthocyanin conjugates along with vanillic acid, ferulic acid, hippuric acid and 4-hydroxybenzaldehyde in urine [6, 12].

Toxicological studies

A number of acute and short-term toxicity studies were identified using anthocyanins from a range of sources, including dried fruits and vegetables and extracts of these. In many cases, the anthocyanins in the test material were not identified or quantified [13–17].

In the acute toxicity studies, no effects were observed at oral test substance doses up to 25 000 mg/kg bw [13–17].

No short-term studies were carried out using black carrot extract. A number of short-term studies in a range of species using test substances containing anthocyanins were identified. No treatment-related effects were observed in a 28-day mouse study using dried red cabbage powder [16], two 90-day studies in rats given grape seed extract [14, 18], one 90-day study in rats given an anthocyanin extract [13], one 90-day study in rats given grape skin extract [18] and two 90-day studies in dogs, one using grape colour powder and one grape skin extract [19, 20]. In addition, no effects were observed in a 15-day study in guinea-pigs given anthocyanins in the diet [13].

In a study in rats fed a diet supplemented with grape skin extract at 0, 2000, 10 000 or 50 000 mg/kg feed (equal to 0, 100, 600 and 3300 mg/kg bw per day for males and 0, 100, 700 and 3600 mg/kg bw per day for females, respectively) for 90 days, the anthocyanin content was not characterized, but the test material was said to contain approximately 2% anthocyanins (anthocyanin doses were therefore 0, 2, 12 and 66 mg/kg bw per day for males and 0, 2, 14 and 72 mg/kg bw per day for females, respectively). In this study, calcification of the proximal tubules of the kidney was identified in females in all dose groups, including controls, but the severity was significantly higher in the group receiving 50 000

mg/kg in the diet. A NOAEL of 14 mg/kg bw per day expressed as anthocyanin (10 000 mg/kg feed expressed as grape skin extract, equal to 700 mg/kg bw per day) was identified [21].

In a study by Nabae et al. [22], in which rats were administered purple corn colour (containing 26.4% cyanidin-3-*O*-glucoside) in the diet at 0, 5000, 15 000 or 50 000 mg/kg feed (equal to cyanidin-3-*O*-glucoside doses of 0, 84, 249 and 935 mg/kg bw per day for males and 0, 89, 272 and 1016 mg/kg bw per day for females, respectively) for 90 days, a number of statistically significant findings were observed at the top dose, including effects on haematological and clinical chemistry parameters and relative organ weights. Although the authors concluded that the NOAEL was the highest dose tested, the Committee was of the opinion that the effects observed at 50 000 mg/kg feed were toxicologically relevant and identified a NOAEL of 15 000 mg/kg feed (equal to 249 mg/kg bw per day).

No long-term toxicity or carcinogenicity studies are available.

Eight in vitro and seven in vivo genotoxicity studies are available, but only one assay (an in vitro comet assay in human colon cancer cells) used black carrot extract as the test material [23]. This study showed positive results only at cytotoxic concentrations. No findings were observed for any of the anthocyanin-containing test materials that would raise concerns for genotoxicity [14, 17, 23–29].

Two multigeneration reproductive toxicity studies are available. One of these used grape colour powder administered to rats in the diet at 0, 7500 or 15 000 mg/kg bw per day, but the anthocyanins in the test material were not quantified. There were no treatment-related findings [20]. In a second study, using a grape skin extract preparation (containing 3% anthocyanins; composition of anthocyanins not given) administered to rats in the diet at a concentration of 0, 75 000 or 150 000 mg/kg feed (equivalent to 0, 7500 and 15 000 mg/kg bw per day, respectively, estimated to be 0, 225 and 450 mg/kg bw per day expressed as anthocyanins), decreases in liver, adrenal and thyroid weights were observed in the top-dose group of the first filial (F₁) generation. The NOAEL for this grape skin extract preparation identified by the previous Committee was 75 000 mg/kg feed (equivalent to 7500 mg/kg bw per day and estimated to be 225 mg/kg bw per day expressed as anthocyanins) [1]. The ADI for anthocyanins from grape skin extract established by the previous Committee was based on this study, with application of an uncertainty factor of 100 to the NOAEL and rounding.

The anthocyanin glycosides (an extract from currants, blueberries and elderberries) were reported not to be teratogenic in rats, mice or rabbits when given at a dose of 1500, 3000 or 9000 mg/kg bw per day over three successive generations [13].

Observations in humans

A number of studies have been carried out in humans to identify biological effects of anthocyanins. Although no toxicity issues have been identified from these studies, the study designs limit their suitability for deriving safe levels of anthocyanins.

Assessment of dietary exposure

In the submission to the Committee, the sponsors proposed the use of black carrot extract as a food colour at typical and maximum use levels (expressed as total anthocyanins in milligrams per kilogram) in 77 food categories and subcategories as specified in the Codex GSFA. The anthocyanin content in black carrot extracts reported by the sponsors ranges from 0.8% to 14.5%, with a standardized content of 9%.

The Committee considered the European estimates of dietary exposure to anthocyanins, provided by the sponsors, as being the most representative of actual exposure. The Committee noted that the mean estimated dietary exposures to total anthocyanins ranged from less than 0.1 mg/kg bw per day for the elderly population up to 1.3 mg/kg bw per day for toddlers. The 95th percentile exposure for consumers only ranged from 0.1 mg/kg bw per day for the elderly population up to 6.9 mg/kg bw per day for toddlers in the brand-loyal scenario, whereas the 95th percentile exposure ranged from less than 0.1 mg/kg bw per day for toddlers and children up to 2.4 mg/kg bw per day for toddlers in the non-brand-loyal scenario. The main foods contributing to the overall exposure to anthocyanins were non-alcoholic beverages, flavoured fermented desserts and cider.

The Committee also considered typical exposure to anthocyanins from natural sources. Anthocyanins are naturally present in foods such as fruits, vegetables, nuts, chocolate, tea and wine. The mean dietary exposure to anthocyanins in the USA using National Health and Nutrition Examination Survey (NHANES) 2001–2002 data [30] was 0.2 mg/kg bw per day for a 60 kg adult. In Europe, the mean dietary exposure to anthocyanins using the Comprehensive European Food Consumption Database [31] ranged from 0.05 mg/kg bw per day for adolescents to 1.6 mg/kg bw per day for adults and up to 4 mg/kg bw per day for toddlers.

The Committee noted that the European dietary exposures to anthocyanins from natural sources as described in the current evaluation are higher than the mean dietary exposure of 0.3 mg/kg bw per day that was reported for Europe by EFSA [32], which at that time was based on one national dietary survey from Europe.

With regard to use levels evaluated at this meeting, the Committee noted differences between the sponsors' reported current and proposed use levels of black carrot extract expressed as total anthocyanins and those that were

considered in the EFSA [32] evaluation. The main difference was for the food category processed meat, which is not proposed as a food to which anthocyanins could be added. In the EFSA [32] evaluation, processed meat was the main food contributing to overall exposure to total anthocyanins, contributing up to 30–50% of the average dietary exposures across Europe (0.5–2.4 mg/kg bw per day).

Evaluation

There are no data on the toxicity of black carrot extract, with the exception of one genotoxicity test. Nevertheless, the Committee noted the large number of studies on other sources of anthocyanins published since anthocyanins were last evaluated by JECFA in 1982, including toxicity studies in animals and ADME studies in humans.

The Committee concluded that the effects observed with one anthocyanin-containing test material cannot be extrapolated to another anthocyanin-containing test material based on the available information. This is because the test articles in the metabolism and toxicity studies evaluated at this meeting were very heterogeneous and often not fully described and/or the anthocyanin content of the test material was too low and variable. This agrees with the conclusion of the previous Committee ([Annex 1](#), reference 59).

Owing to the lack of toxicological data on black carrot extract, the Committee was not able to draw conclusions on its safety. To proceed with the assessment of black carrot extract, at least a 90-day toxicological study on a well-characterized extract representative of the material of commerce would be required.

The Committee concluded that the total mean dietary exposure to anthocyanins from naturally occurring sources and added black carrot extract using the non-brand-loyal scenario ranges from 0.1 to 1.9 mg/kg bw per day for the adult population (18+ years old) and from 0.1 to 5.3 mg/kg bw per day for children (<18 years old).

In these estimates, the Committee noted that the use of black carrot extract itself as proposed by the sponsors contributes as much as 25% to the total mean dietary exposure to anthocyanins, including from naturally occurring sources. The Committee noted that the ADI for grape skin extract established by the previous Committee in 1982 was not reconsidered as part of this assessment and remains unchanged.

A consolidated toxicological and dietary exposure monograph was prepared.

At the present meeting, new specifications for the spray-dried powder form of black carrot extract were prepared. The specifications were made tentative pending the submission of further information on the material of commerce (see [Recommendations](#) below).

A Chemical and Technical Assessment was prepared.

Recommendations

To proceed with the assessment of black carrot extract, at least a 90-day toxicological study on a well-characterized extract representative of the material of commerce would be required.

The specifications were made tentative pending the submission of further information on the material of commerce, including a full characterization of the proteins, carbohydrates, lipids, fibre, minerals and non-anthocyanin polyphenol components in five lots each of the liquid and powder forms of black carrot extract.

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3.1.2 Brilliant Black PN

Explanation

Brilliant Black PN (INS 151; Chemical Abstracts Service [CAS] No. 2519-30-4) is a synthetic disazo dye used as a food colouring agent. JECFA first evaluated Brilliant Black PN at its eighteenth meeting ([Annex 1](#), reference 35) and established a temporary ADI of 0–2.5 mg/kg bw, based on a NOAEL of 500 mg/kg bw per day obtained from a chronic rat study. An additional uncertainty factor of 2 was applied because the ADI was temporary, pending the submission of metabolic, reproductive and embryotoxicity studies.

At the twenty-second meeting of JECFA ([Annex 1](#), reference 47), the requested metabolic, reproductive and embryotoxicity studies were not submitted. In addition, the Committee indicated that the etiology and pathology of ileal cysts observed in a 90-day toxicity study in pigs submitted at that meeting should be determined. The Committee maintained the temporary ADI.

At the twenty-fifth meeting of JECFA ([Annex 1](#), reference 56), multigeneration reproductive toxicity and teratogenicity studies were submitted, both showing no toxicologically relevant effects. A metabolic study was also submitted. No further information on the ileal cysts in pigs was available. Therefore, the Committee established a new ADI of 0–1 mg/kg bw on the basis of the no-effect level of 100 mg/kg bw per day in the pig study.

Brilliant Black PN was placed on the agenda of the present meeting for re-evaluation of its safety, evaluation of its dietary exposure and revision of its specifications, at the request of the Forty-ninth Session of CCEFA [1].

Studies on the effects of Brilliant Black PN on enzymes and other biochemical parameters, genotoxicity studies, studies on the toxicity of metabolites and a study on non-allergic hypersensitivity in children were submitted. Additional literature searches in Medline, Toxline, Scopus and SciFinder using the keywords Brilliant Black, clinical, toxicology, genotoxicity, metabolism, absorption, excretion and ADME did not identify any additional

relevant publications. The sponsor submitted use levels of Brilliant Black PN in three main food categories as well as dietary exposure estimates reported in the literature.

Chemical and technical considerations

Brilliant Black PN is intended for use in colouring confectionery, decorations and coatings, desserts including flavoured milk products, edible cheese rind, edible ices, fine bakery wares, fish and fish products, non-alcoholic flavoured drinks, non-dairy beverages, sauces and seasonings, and savoury snacks.

Brilliant Black PN consists mainly of tetrasodium 4-(acetylamino)-5-hydroxy-6-[2-[7-sulfo-4-[2-(4-sulphophenyl)diazenyl]-1-naphthalenyl]diazenyl]-1,7-naphthalenedisulfonate and subsidiary colouring matters. Sodium chloride and/or sodium sulfate are the principal uncoloured components. Brilliant Black PN is manufactured by diazotizing 4-aminobenzenesulfonic acid (sulfanilic acid), coupling with 8-aminonaphthalene-2-sulfonic acid (1,7-Cleve's acid), diazotizing the product and coupling with 4-(acetylamino)-5-hydroxy-1,7-naphthalenedisulfonic acid (*N*-acetyl K acid). The dye is isolated as the tetrasodium salt. Impurities include unreacted starting materials and reaction by-products ($\leq 0.8\%$), subsidiary colouring matters ($\leq 4\%$), unsulfonated primary aromatic amines ($\leq 0.01\%$ calculated as aniline) and lead (≤ 2 mg/kg).

Biochemical aspects

In rats, Brilliant Black PN is poorly absorbed, with 94–98% of administered doses up to 10 mg/kg bw excreted in the faeces within 40 hours and less than 5% detected in the urine within 40 hours [2]. Differences in metabolism following oral and intraperitoneal administration indicate that metabolism by intestinal flora leads to complete azo reduction (cleavage of both azo sites), whereas azoreductases in liver preferentially cleave the azo site between the two naphthalene rings, resulting in sulfonated aromatic amines [3].

In humans, sulfanilic acid was the only metabolite identified in urine following oral administration of a 240 mg dose of Brilliant Black PN, and the amount of metabolite was similar to that observed in rats [3].

In vitro, Brilliant Black PN was shown to induce a dose-dependent decrease in the uptake of radiolabelled *o*-iodohippurate and iodipamide in rat renal cortex slices, which was interpreted as inhibition of the hippurate and liver-like anion transport systems [4].

Brilliant Black PN was identified as a novel allosteric modulator of adenosine receptors using Chinese hamster ovary cells stably transfected with either A₁ or A₃ human receptors [5]. The Committee noted that the effects on adenosine receptors were observed only at high (500 $\mu\text{mol/L}$) concentrations

of Brilliant Black PN. In view of the poor absorption of Brilliant Black PN, the Committee did not consider this study relevant to the evaluation.

Toxicological studies

In previously evaluated studies, Brilliant Black PN was not acutely toxic by the oral route in mice or rats (median lethal dose [LD_{50}] > 5000 mg/kg bw) [6, 7] and showed no signs of toxicity in mice in a long-term study at doses up to 1300 mg/kg bw per day [8] or in rats in short-term studies with dietary concentrations up to 30 000 mg/kg feed (equivalent to 3000 mg/kg bw per day) and long-term studies with dietary concentrations up to 10 000 mg/kg feed (equal to 360 mg/kg bw per day) [7, 9] and no evidence of carcinogenicity in mice or rats [8, 9]. In rats, there was no reproductive toxicity or teratogenicity at dietary concentrations up to 30 000 mg/kg feed (equivalent to 1500 mg/kg bw per day) [10] and no teratogenicity at doses up to 2500 mg/kg bw per day [11].

The only adverse findings reported previously were cysts containing mucus and fibrin in the ileal mucosa of pigs administered Brilliant Black PN at 300 mg/kg bw per day (one of six pigs) or 900 mg/kg bw per day (four of six pigs) for 90 days. The NOAEL in this study was 100 mg/kg bw per day [12]. The Committee at the twenty-fifth meeting ([Annex 1](#), reference 56) established an ADI based on this NOAEL. In the current submission, the sponsor reiterated the authors' argument that the cysts might have been due to an irritant effect of local high concentrations of Brilliant Black PN related to the way in which the substance was administered as a bolus in a small amount of feed. The present Committee considered that this explanation lacked plausibility, as the upper parts of the gastrointestinal tract were not affected, as would be anticipated for an irritant effect.

Several new in vitro genotoxicity studies [13–15] and one new in vivo genotoxicity study [13] were available to the Committee and were generally negative. The gene mutation assay in mammalian cells [15] was equivocal in the presence of metabolic activation, which normally would require follow-up, and aneugenicity was not tested for. The positive findings obtained in the in vitro micronucleus test and comet assay are considered to be unreliable due to major shortcomings in study design. Read-across from a structurally related food colour (Allura Red AC) [16] and the lack of genotoxicity of other sulfonated aromatic amines such as those generated by the azoreduction of sulfonated azo dyes [17] were taken into consideration. The Committee concluded that, overall, the data did not indicate concern with respect to the genotoxicity of Brilliant Black PN.

The metabolite sulfanilic acid (the only metabolite found in human urine) did not show genotoxic activity or adverse effects in a 4-week study or in an Organisation for Economic Co-operation and Development (OECD)–

Table 1
Dietary exposure to Brilliant Black PN

Country/region	Dietary exposure (mg/kg bw per day)	
	Mean	High
Australia ^a		
Mean	0.00–0.001	0.00–0.002 ^b
Maximum	0.00–0.004	0.003–0.01 ^b
Europe ^c	0.01–0.17	0.02–0.30 ^d
Kuwait ^e	0.000 2–0.000 3	–

^a Exposure only for consumers of foods containing Brilliant Black PN based on mean and maximum analytical concentrations.

^b Ninetieth percentile of exposure.

^c Non-brand-loyal scenario based on mean use levels and analytical concentrations.

^d Ninety-fifth percentile of exposure.

^e Children; based on analytical concentrations.

Sources: Australia: [22, 23]; Europe: [24]; Kuwait: [25]

compliant reproductive and developmental toxicity study in rats administered doses up to 1000 mg/kg bw per day [18, 19].

Observations in humans

A study in six young patients with moderate to severe chronic urticaria found that one child exhibited immunoglobulin E–independent responses to all tested azo dyes, including Brilliant Black PN [20]. The Committee noted that this study is not informative for the present evaluation.

Assessment of dietary exposure

Brilliant Black PN is proposed by the sponsor for use in 16 food subcategories belonging to three main food categories of the Codex GSFA: “5. Confectionery”, “9. Fish and fish products, including mollusks, crustaceans, and echinoderms” and “14. Beverages, excluding dairy products”. The typical use levels range from 10 to 300 mg/kg, and the maximum use levels from 10 to 500 mg/kg. Currently, Brilliant Black PN is authorized for use only in food category “01.1.4 Flavoured fluid milk drinks”, excluding chocolate milk, at a maximum permitted level of 150 mg/L, as specified in the GSFA [21].

The Committee used only those dietary exposure estimates that were considered to be most representative of actual exposure. These estimates were based on use levels and/or analytical concentrations combined with food consumption data from Australia [22, 23], Europe [24] and Kuwait [25] and are listed in [Table 1](#).

The dietary exposures to Brilliant Black PN in Australia and Kuwait were estimated using analytical concentrations measured in relevant foods, which resulted in low exposure estimates, as Brilliant Black PN was present in only

a limited number of food groups at low levels [22, 23, 25]. The high exposure (90th percentile) to Brilliant Black PN was maximally 0.01 mg/kg bw per day for children up to 16 years of age in Australia, based on the highest levels analysed per food group [23]. This dietary exposure estimate refers to the exposure in persons who had consumed at least one of the foods that contained Brilliant Black PN (consumers only).

For Europe, the dietary exposure was estimated for different age groups using food consumption data from several European countries combined with maximum permitted levels, use levels and/or analytical concentrations, according to three exposure scenarios [24]. Given that the dietary exposure estimates for Australia and Kuwait were so low, the Committee considered the non-brand-loyal scenario to best reflect the dietary exposure to Brilliant Black PN. In this scenario, it is assumed that persons are exposed to a food additive at the typical (mean) reported use level or mean of the analytical concentrations for all relevant food categories and that all foods belonging to an authorized food category contain the food additive at that level. The mean dietary exposure to Brilliant Black PN ranged from 0.01 mg/kg bw per day for adolescents, adults 18–64 years of age and adults 65+ years of age to 0.17 mg/kg bw per day for toddlers in this scenario. The high dietary exposure (95th percentile) ranged from 0.02 mg/kg bw per day for adults 65+ years of age to 0.30 mg/kg bw per day for toddlers. The food categories included in this scenario overlapped largely with those for which use levels are proposed by the sponsor, in addition to other food categories for which no use levels were proposed, such as “Edible ices”, “Fine bakery wares”, “Seasonings and condiments”, “Soup and broths”, “Mustard” and “Potato-, cereal-, flour- and starch-based snacks”. The Committee noted that the food category “Fine bakery wares” was the most important contributor to the dietary exposure to Brilliant Black PN across all age groups in Europe.

The Committee concluded that the high dietary exposure to Brilliant Black PN of 0.3 mg/kg bw per day, based on European data, is appropriate for use in a risk assessment.

Evaluation

The Committee concluded that the newly available information does not give reason to revise the previously established ADI of 0–1 mg/kg bw based on the short-term toxicity study in pigs. The Committee therefore retained the ADI for Brilliant Black PN.

The Committee noted that the range of estimated dietary exposures for Brilliant Black PN was below the upper end of the ADI and concluded that dietary exposure to Brilliant Black PN does not present a safety concern.

A consolidated toxicological and dietary exposure monograph was prepared.

At the present meeting, the specifications for Brilliant Black PN were revised. Analytical methods for determining subsidiary colouring matters and organic compounds other than colouring matters were replaced with more specific and sensitive HPLC methods. The existing titrimetric method for the assay of Brilliant Black PN was replaced with a visible spectrophotometric method.

A Chemical and Technical Assessment was prepared.

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3.1.3 Carotenoids (provitamin A)

Explanation

β -Carotene (CAS No. 7235-40-7) and β -apo-8'-carotenal (CAS No. 1107-26-2) are provitamin A carotenoids that are used as colours in a wide range of foods and beverages. Currently, both food additives are authorized for use in 79 food categories at maximum permitted levels ranging from 50 mg/kg up to 1200 mg/kg as specified in the Codex GSEA [1].

A group ADI of 0–5 mg/kg bw for β -carotene, β -apo-8'-carotenal and β -apo-8'-carotenoic acid methyl and ethyl esters was first established at the tenth JECFA meeting (Annex 1, reference 13). At its eighteenth meeting, the Committee considered additional data and reaffirmed the decision of the tenth

meeting (Annex 1, reference 35). The group ADI was derived using a four-generation study in rats with a NOAEL for β -carotene of 50 mg/kg bw per day and application of an uncertainty factor of 10 because of the natural occurrence of provitamin A carotenoids in the human diet and the low toxicity observed in animal studies.

β -Carotenes from natural sources were reviewed at the thirty-first, thirty-fifth and forty-first meetings of the Committee (Annex 1, references 77, 88 and 107). At the thirty-first meeting, the Committee concluded that the group ADI of 0–5 mg/kg bw established for the sum of the synthetic carotenoids β -carotene, β -apo-8'-carotenal and β -apo-8'-carotenoic acid methyl and ethyl esters by the eighteenth Committee was not applicable to natural carotenes as they did not comply with the specifications for β -carotene. At the thirty-fifth and forty-first meetings, the Committee considered the available data inadequate to establish an ADI for the dehydrated algal carotene preparations or for the vegetable oil extract of *Dunaliella salina*.

At the fifty-seventh meeting (Annex 1, reference 154), the Committee undertook a re-evaluation of β -carotene for use as a food colour, but focused its assessment on the production and analytical characteristics of β -carotene produced from *Blakeslea trispora*. The Committee considered that the source organisms, the production process and the composition of β -carotene from *B. trispora* do not raise specific concerns and that the material should be considered toxicologically equivalent to chemically synthesized β -carotene, for which an ADI of 0–5 mg/kg bw was established by the Committee at its tenth meeting. Therefore, the Committee established a group ADI of 0–5 mg/kg bw for synthetic β -carotene and β -carotene derived from *B. trispora*.⁴

β -Carotene-rich extract from *D. salina* was evaluated at the eighty-fourth meeting (Annex 1, reference 234). The Committee observed that data that had become available since the previous evaluation showed differences in absorption of β -carotene between rodent species and humans. The Committee considered that rodents were inappropriate animal models for establishing an ADI for β -carotene because of the virtual absence of systemic absorption of β -carotene in rodents, but that the non- β -carotene components of *D. salina* d-limonene extract could be evaluated using the results of rodent studies. The Committee recommended that the group ADI for the sum of carotenoids, including β -carotene, β -apo-8'-carotenal and β -apo-8'-carotenoic acid methyl and ethyl esters, be re-evaluated in light of evidence that shows very low absorption of β -carotene in rodents and rabbits in contrast to humans.

⁴ The present Committee was aware that two group ADIs for carotenoids had been established at previous meetings and that synthetic β -carotene had been included in both group ADIs. The Committee speculated that the Committee at the fifty-seventh meeting did not recognize that synthetic β -carotene was already part of a group ADI and included it in a new group ADI.

β -Carotene, β -apo-8'-carotenal, β -carotene from *B. trispora* and β -apo-8'-carotenoic acid methyl and ethyl esters were placed on the agenda of the present meeting for an assessment of their safety, dietary exposure and specifications in response to the recommendation of the eighty-fourth meeting of the Committee. The present Committee considered a submission that comprised a review of information on synthetic β -carotene and β -apo-8'-carotenal that had become available since the eighteenth meeting. A targeted literature search was additionally carried out.

The Committee noted that no data were submitted on β -apo-8'-carotenoic acid methyl and ethyl esters. These food colours were therefore removed from the agenda.

Chemical and technical considerations

Provitamin A and xanthophyll carotenoids are natural pigments that are synthesized by plants and are responsible for the bright colours of various fruits and vegetables. Many different carotenoids are present in foods, and most have antioxidant activity. The most abundant carotenoid, β -carotene, consists of a highly branched, unsaturated chain with identical substituted ring structures at each end. β -Carotene and β -apo-8'-carotenal are provitamin A carotenoids.

β -Carotene, synthetic

Commercially available β -carotene, synthetic (INS 160a(i)) may be synthesized via a double Wittig condensation process or Grignard synthesis with enol ether condensations using a range of vitamin A precursors, including their phosphonium salts. The products of commerce may exist in multiple formulations, including water-dispersible forms, those that are water soluble and microcrystals prepared by spray drying and bound to food-grade carriers and antioxidants. Solvents used in manufacture may include dichloromethane, hexane, methanol, methylcyclohexane, toluene, acetone, ethanol, ethyl acetate, heptane, isobutyl alcohol and isopropyl alcohol [2]. The colouring principle of β -carotene, synthetic consists predominantly of all-*trans*- β -carotene (*E*-isomer) together with minor amounts of other carotenoids. The total colouring matters content is not less than 96% (expressed as β -carotene).

β -Carotene from *Blakeslea trispora*

β -Carotene from *Blakeslea trispora* (INS 160a(iii)) is obtained by co-fermentation using a mixed culture of the two sexual mating types (+) and (-) of natural strains of the fungus that are non-pathogenic and non-toxicogenic. The compound is isolated from the fungal biomass by solvent extraction and crystallized. The main articles of commerce are suspensions in food-grade vegetable or plant oil and water-dispersible powders. These formulations are made for ease of use and

in order to improve stability, as carotenes easily oxidize. β -Carotene from *B. trispora* may also contain other carotenoids, of which λ -carotene accounts for the major part, at concentrations up to 3%. As in synthetically produced β -carotene, the colouring principle of β -carotene from *B. trispora* consists predominantly of all-*trans*- β -carotene. The total colouring matters content is not less than 96% (expressed as β -carotene).

β -Apo-8'-carotenal

β -Apo-8'-carotenal (INS 160a(vi)) occurs naturally in various plant materials as an aldehydic carotenoid. The product of commerce is synthetically produced using multiple mechanisms that may include the use of vitamin A precursor molecules and Wittig-type condensation reactions. Sequential chemical reactions are carried out to produce the final material, which exists predominantly as the all-*trans* (*E*) isomer. The articles of commerce may be diluted and stabilized as suspensions in edible fats or oils, emulsions and water-dispersible powders. The total colouring matters content is not less than 96%.

β -Carotene-rich extract from *Dunaliella salina*

β -Carotene-rich extract from *Dunaliella salina* is produced from *D. salina*, an extremely halotolerant alga that inhabits natural and human-made salt lakes and ponds. The carotene-rich alga is harvested and concentrated, and the carotenoids are extracted using an essential oil rich in d-limonene. The resulting extract is saponified, purified, centrifuged, evaporated and finally mixed with a vegetable oil to obtain a commercial product with a carotene content of about 30% by weight. β -Carotene accounts for more than 95% of the carotene content of the extracted material as a mixture of *trans* and *cis* isomers in a ratio of approximately 2:1 by weight. The remainder of the carotene content includes α -carotene, lutein, zeaxanthin and cryptoxanthin. In addition to the colour pigments and vegetable oil used for standardization, d-limonene extracts of *D. salina* contain lipids and other fat-soluble components naturally occurring in the source material, such as fatty acids, long-chain alcohols, alkenes and waxes. The composition of these fat-soluble components is primarily a mixture of fatty acids common to vegetable oils used in foods.

Biochemical aspects

β -Carotene

β -Carotene is absorbed into enterocytes and centrally cleaved to give two retinal molecules. Retinal is reduced to retinol by the enzyme retinaldehyde reductase and then esterified to form retinyl esters by lecithin:retinol acyltransferase and packaged with chylomicrons. Chylomicrons containing retinyl esters are released

into the lymph and then the bloodstream and rapidly taken up into the liver [reviewed in 3, 4]. Although the mechanism of intestinal β -carotene absorption and metabolism appears to be comparable in animal models and humans, marked differences in cleavage rates and consequently bioavailability between species have been shown [5–10].

In a short-term toxicity study in rats administered β -carotene at a dose of 0, 250, 500 or 1000 mg/kg bw per day for 13 weeks, plasma β -carotene concentrations ranged from 0.4 to 0.9 $\mu\text{g/mL}$ [11]. In other studies in rats administered β -carotene at doses up to 1000 mg/kg bw per day for up to 21 weeks, plasma β -carotene concentrations ranged from below the limit of detection to about 0.2 $\mu\text{g/mL}$ [12–14]. More than 95% of radioactivity in plasma and approximately 88–94% of radioactivity in liver were identified as retinol in rats administered 0.5 mg (0.74 MBq) radiolabelled β -carotene. β -Carotene was not detected in plasma [15].

In human subjects, the absorption of β -carotene has been estimated to be in the range of 40–65% [16–18]. In human subjects administered radiolabelled β -carotene, radioactivity in lymph was mainly associated with chylomicrons as retinyl esters, with approximately 20–30% of the absorbed radioactivity recovered as β -carotene [19, 20]. Following the administration of ^{13}C -labelled β -carotene to humans, most of the absorbed dose was converted to vitamin A [21]. Excretion of radioactivity occurred mainly via the faeces, with smaller amounts in the urine [16–18].

A number of studies in human subjects also investigated plasma levels of β -carotene following dosing for up to 12 years with pharmacological amounts of β -carotene. The most informative of these were a number of randomized controlled trials. Mean or median plasma β -carotene levels increased from 0.3 to 1.2 $\mu\text{g/mL}$ in subjects administered 50 mg β -carotene every second day [22]; from 0.17 to 3.0 $\mu\text{g/mL}$ in subjects administered 20 mg β -carotene per day [23]; and from 0.15 to 2.1 $\mu\text{g/mL}$ in subjects administered 30 mg β -carotene per day with 25 000 IU vitamin A [24, 25].

Based on the observed differences in cleavage rates and bioavailability of β -carotene between rats and humans, the Committee reaffirmed the conclusion of the eighty-fourth meeting that this species is not suitable for the evaluation of β -carotene in humans. Absorption and tissue disposition studies with β -carotene in mice or dogs were not available to the Committee.

β -Apo-8'-carotenal

Radiolabelled β -apo-8'-carotenal and its metabolites were at least 25% absorbed from the gastrointestinal tract of rats. Total radioactivity in plasma reached a peak concentration after 10 hours and was eliminated with a half-life of 21 hours. β -Apo-8'-carotenal and its metabolites β -apo-8'-carotenol, β -apo-8'-carotenoic

acid and fatty acid conjugates were identified in the plasma. Radioactivity was recovered in the liver as retinol and fatty acid conjugates of retinol, demonstrating conversion of β -apo-8'-carotenal to vitamin A. Elimination of radioactivity occurred mainly via faeces, with smaller amounts excreted in the urine [26].

A clear sex-related difference was seen in a 13-week toxicity study in which female rats showed higher concentrations of β -apo-8'-carotenal and/or its metabolites in the plasma and liver compared with males [27].

β -Apo-8'-carotenal did not appear in plasma in significant amounts in human male volunteers given a single oral dose of 41 mg β -apo-8'-carotenal. β -Apo-8'-carotenol and β -apo-8'-carotenyl palmitate were identified as the two major metabolites in the plasma and reached their maximum concentrations of 0.29 and 0.23 $\mu\text{mol/L}$ at 11 and 6 hours, respectively. 3-Apo-8'-carotenoic acid was also detected in serum, but the concentrations were not determined [28].

Toxicological studies

β -Carotene

β -Carotene has low acute oral toxicity in rats and dogs [29–32].

No target organ toxicity was observed in short- or long-term studies in rats or dogs administered β -carotene [11, 33–37].

β -Carotene was not carcinogenic in mice or rats [33, 35].

β -Carotene was not genotoxic in vitro or in vivo [32, 38–40].

There was no evidence of reproductive or developmental toxicity in studies in rats or rabbits [41–43].

β -Apo-8'-carotenal

β -Apo-8'-carotenal has low acute oral toxicity in mice [44] and rats [45, 46].

Two new short-term studies in rats were available to the Committee. In a 28-day study, rats were given β -apo-8'-carotenal in the feed at a target dose of 0, 20, 100 or 500 mg/kg bw per day. A NOAEL of 100 mg/kg bw per day was established on the basis of reduced body weight and body weight gain in rats at 500 mg/kg bw per day [47]. The Committee noted the presence of eosinophilic droplets mainly in the kidneys of female rats, but did not consider the finding to be adverse on the basis that the droplets were not linked to other lesions or any other signs of nephropathy.

In a follow-up 90-day study, male and female rats were administered β -apo-8'-carotenal in the feed at a target dose of 0, 10, 30 or 100 mg/kg bw per day. Liver weight and serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were significantly increased in female rats at the high dose relative to controls. Upon histopathological examination, a significant increase in the incidence of inflammatory cell foci was seen in the liver of female

rats at 100 mg/kg bw per day. In the kidney, an increase in the incidence of minimal eosinophilic droplets was observed in females at all doses, increasing in severity in high-dose females, but there was no evidence of necrosis or single-cell death at any dose. Findings of tubular injury were generally limited to the occasional tubular epithelial cell containing eosinophilic material appearing to be detached from the tubule and the presence of mitotic figures in the cortex in males and females at 100 mg/kg bw per day [27]. The Committee identified a NOAEL of 30 mg/kg bw per day, on the basis of increased liver weight, serum ALT and AST activities and incidence of inflammatory cell foci in the liver of high-dose female rats and evidence of tubular injury in the kidney of high-dose males and females. The Committee noted that higher plasma and liver concentrations of β -apo-8'-carotenal or its metabolites were achieved in female rats compared with males and considered this to be consistent with the toxicological findings of this study.

No new long-term toxicity or carcinogenicity studies were available to the Committee. In an early study, rats were administered β -apo-8'-carotenal in the diet at 1000 mg/kg feed to (1) a first generation of rats for 2 years, (2) their offspring for 2 years and (3) a third generation of rats for 1 year. The average dose over the course of the study was reported to be 40 mg/kg bw per day. Histopathological examination of the liver and kidneys of treated animals did not identify any adverse effects [48].

β -Apo-8'-carotenal was not mutagenic in *Salmonella typhimurium* strain TA98, TA100, TA102, TA1535 or TA1537 at concentrations of 8.7–277.9 μ g/plate, with or without metabolic activation [49]. In an earlier study, conducted with β -apo-8'-carotenal of low purity (69.2%), a concentration-related increase in the number of revertants was observed only in strain TA100, in the absence and presence of metabolic activation. The study authors noted that this result could be associated with impurities in the test material [50]. In an in vitro mammalian chromosomal aberration test, an increased frequency of chromosomal aberrations was observed in the absence and presence of metabolic activation at concentrations associated with approximately 50% cytotoxicity or greater, but not at lower concentrations, where moderate to low cytotoxicity was observed. The study authors noted that these increases were of questionable biological relevance given that they were observed only at doses associated with significant cytotoxicity [51]. In an in vivo mammalian erythrocyte micronucleus test, treatment with β -apo-8'-carotenal did not induce statistically significant increases in the frequency of micronucleated erythrocytes compared with the concurrent controls at doses up to 800 mg/kg bw per day [52]. The Committee concluded that the weight of evidence suggests that there is no concern for genotoxicity of β -apo-8'-carotenal.

No reproductive toxicity studies were available to the Committee. In a good laboratory practice- and guideline-compliant developmental toxicity study,

rats were administered β -apo-8'-carotenal in the feed from gestation days 6 to 20 at a dose of 0, 20, 100 or 495 mg/kg bw per day. No maternal or developmental toxicity was observed. The NOAEL for maternal toxicity and for embryo and fetal toxicity was 495 mg/kg bw per day, the highest dose tested [53].

Observations in humans

The association between β -carotene intake and cancer risk has been evaluated in a number of observational studies and extensively reviewed [9, 54–56]. It was concluded that intake of β -carotene and fruits and vegetables appears to confer protection against cancers at different sites, with the most consistent effect being a protective effect against lung cancer. Consequently, a number of large, high-quality randomized controlled trials have investigated whether β -carotene supplementation at doses of 20–50 mg/day for durations of up to 12 years reduces cancer risk in human populations.

In the Alpha-Tocopherol, Beta Carotene Cancer Prevention (ATBC) Study, a higher incidence of lung cancer (relative risk [RR] 1.18; 95% confidence interval [CI] 1.03–1.36) and total mortality (RR 1.08; 95% CI 1.01–1.16) was observed among the men who received β -carotene at doses of 20 mg/day for between 5 and 8 years. The elevated risk was related to those who smoked at least one pack of cigarettes per day and was not seen in subjects who smoked less [9, 23]. In the Beta-Carotene and Retinol Efficacy Trial (CARET), participants who were smokers or ex-smokers, or were exposed to asbestos, were given daily doses of 30 mg β -carotene and 25 000 IU vitamin A as retinyl palmitate for 5 years. Lung cancer incidence and total mortality were increased by 28% (RR 1.28; 95% CI 1.04–1.57) and 17% (RR 1.17; 95% CI 1.03–1.33), respectively, in the supplemented group [24, 25].

The Committee noted that the effects observed in heavy smokers and asbestos workers in the ATBC and CARET studies were not seen in population subgroups that were not at increased risk of lung cancer. In the Physicians' Health Study, β -carotene administered to subjects at 50 mg every second day for a period of 12 years did not affect the number of cases of lung cancer, mortality from cancer, all-cause mortality, cardiovascular disease, myocardial infarction or stroke [22]. No effects on cancer incidence or total mortality were seen in a number of other smaller randomized controlled trials in which β -carotene was administered at doses of up to 50 mg/day for durations of up to approximately 9 years [57–62].

Assessment of dietary exposure

β -Carotene and β -apo-8'-carotenal are proposed by the sponsor for use at typical and maximum use levels in 33 and 12 food categories of the Codex GSFA, respectively. For β -carotene, the typical (mean) and maximum use levels ranged

Table 2

Overview of dietary exposure estimates for β -carotene and β -apo-8'-carotenal from their use as food additives in the European population

Source	Dietary exposure (mg/kg bw per day)			
	β -Carotene		β -Apo-8'-carotenal	
	Mean	High	Mean	High
EFSA ^a	0.03–0.22	0.09–0.43 ^b	0.01–0.25	0.04–0.49 ^c
EFSA FAIM ^d	0.02–0.19	0.03–0.28 ^c	–	–
France, Germany, United Kingdom ^{e,f}	0.009	0.03 ^g	–	–

bw: body weight; EFSA: European Food Safety Authority; FAIM: Food Additive Intake Model

^a All age groups; maximum use levels.

^b High exposure: 95th or 97.5th percentile.

^c High exposure: 95th percentile.

^d All age groups; typical (mean) use levels.

^e Adults; typical (mean) use levels.

^f Exposure estimated by the present Committee using a 60 kg adult body weight.

^g High exposure: 97.5th percentile.

Sources: EFSA: [2, 63]; EFSA FAIM (sponsor submission); France, Germany, United Kingdom: [64]

from 1 to 20 mg/kg and from 2 to 70 mg/kg, respectively. Corresponding ranges for β -apo-8'-carotenal were 0.4–50 mg/kg and 0.4–260 mg/kg. Currently, both food additives are authorized for use in 79 food categories at maximum permitted levels ranging from 50 mg/kg up to 1200 mg/kg, as specified in the GSFA [1].

The Committee used the exposure estimates submitted by the sponsor, which more closely represent actual exposure. These estimates were based on use levels combined with food consumption data from Europe [2, 63] and on a study on dietary exposure to β -carotene based on food consumption data from France, Germany and the United Kingdom [64]. Furthermore, the sponsor also reported on the exposure to β -carotene calculated with the EFSA Food Additive Intake Model (FAIM). The dietary exposure estimates are listed in Table 2.

The Committee concluded that the exposure to β -carotene from its use as a food additive at typical (mean) use levels estimated with EFSA FAIM is appropriate for use in risk assessment. The upper level of 0.28 mg/kg bw per day refers to the exposure in children aged 1–9 years. For adults aged 18 and above, the upper level of exposure to β -carotene equals about 0.1 mg/kg bw per day. The Committee acknowledged that these dietary exposure estimates were overestimations due to the assumption that β -carotene is used in all foods belonging to the relevant food categories.

The Committee considered that the high daily exposure estimate for β -apo-8'-carotenal of 0.49 mg/kg bw per day overestimates the exposure to this additive, owing to the assumption that all foods contained the food additive at the maximum use level. The Committee therefore concluded that the high daily

dietary exposure to β -carotene of 0.28 mg/kg bw per day may also be used for risk assessment of β -apo-8'-carotenal.

Evaluation

The Committee reaffirmed the conclusion from the eighty-fourth meeting that rats are not an appropriate model for deriving an ADI for β -carotene due to the relatively low bioavailability of β -carotene in rats compared with humans. Therefore, the Committee withdrew the two group ADIs of 0–5 mg/kg bw for (1) the sum of the synthetic carotenoids β -carotene, β -apo-8'-carotenal and β -apo-8'-carotenoic acid methyl and ethyl esters and (2) synthetic β -carotene and β -carotene derived from *Blakeslea trispora*, which were based on a NOAEL from a rat study.

The Committee considered that no adverse health effects were observed in the general population in large, well-conducted human intervention studies in which healthy participants were administered between 20 and 50 mg β -carotene per day for up to 12 years, in addition to the background exposure from the diet.

An additional elevated risk of lung cancer and total mortality was seen in heavy smokers (at least one pack per day) and asbestos workers in intervention studies in which participants were administered 20 mg β -carotene per day for 5–8 years or 30 mg β -carotene per day and 25 000 IU vitamin A for 5 years. The Committee noted that a generally accepted explanation for the cause of these effects has not been identified. The Committee was unable to reach any conclusion about risk from β -carotene exposure in heavy smokers.

For the remainder of the general population, the Committee concluded that the estimated high exposure to β -carotene at 9 mg/day for a 30 kg child and 6 mg/day for a 60 kg adult from its current uses as a food additive, in addition to background exposure from the diet, would not be expected to be a safety concern. This conclusion includes synthetic β -carotene, β -carotene derived from *B. trispora* and β -carotene-rich extract from *Dunaliella salina*.

The Committee was unable to establish a group ADI for synthetic β -carotene, β -carotene derived from *B. trispora*, β -carotene-rich extract from *D. salina*, and β -apo-8'-carotenoic acid methyl and ethyl esters because a group ADI is applicable to the general population, which includes heavy smokers. The Committee noted that it is very unlikely that it will ever be possible to establish a group ADI because further data from the heavy smoker population cannot be gathered ethically.

Because β -apo-8'-carotenoic acid methyl and ethyl esters were previously evaluated on the basis of β -carotene and because no new data were submitted, the Committee was unable to complete an evaluation on β -apo-8'-carotenoic acid methyl and ethyl esters.

The present Committee established an ADI of 0–0.3 mg/kg bw for β -apo-8'-carotenal on the basis of a NOAEL of 30 mg/kg bw per day in a 13-week study in rats and application of an uncertainty factor of 100. An additional uncertainty factor to take into account the short duration of the study was not considered necessary because renal injury and hepatic lesions observed in the 13-week study at 100 mg/kg bw per day were not observed in the 2-year study at 40 mg/kg bw per day, the single dose tested.

Estimated dietary exposure to β -apo-8'-carotenal of 0.28 mg/kg bw per day was at the upper end of the ADI established by the Committee (i.e. 0–0.3 mg/kg bw). The Committee noted that the estimated dietary exposure is overestimated and concluded that the current use of β -apo-8'-carotenal as a food additive will not pose a safety concern.

A toxicological and dietary exposure monograph was prepared.

The specifications for β -carotene, synthetic, β -carotene from *B. trispora* and β -apo-8'-carotenal were revised to replace an identification test for carotenoids with additional spectrophotometric requirements.

β -Carotene-rich extract from *D. salina* was on the agenda of the current meeting at the request of the Fiftieth Session of CCFA [65] to revise the maximum limit on arsenic. The Committee received sufficient analytical data. Based on the arsenic levels from several batches of the product of commerce, the existing specifications were revised from 1 mg/kg to 3 mg/kg. The Chemical and Technical Assessment was revised.

Recommendations

The Committee noted that the use levels of β -carotene and β -apo-8'-carotenal provided by the sponsor were much lower than the corresponding maximum permitted levels as specified in the GSFA, and that the sponsor indicated that the majority of the maximum permitted levels are not justifiable from a technological point of view. Also, use levels were not provided for all authorized food categories. The Committee recommended that the Codex Alimentarius Commission should review current uses of β -carotene (synthetic β -carotene, β -carotene from *B. trispora* and β -carotene-rich extract from *D. salina*) and β -apo-8'-carotenal in the GSFA, including the maximum permitted levels and the food categories in which these food additives may be used.

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3.1.4 Gellan gum

Explanation

Gellan gum (INS 418; CAS No. 71010-52-1) is used as a gelling agent, stabilizer and thickener in a wide range of foods and beverages listed in the Codex GSFA, under the conditions of good manufacturing practice. It is commercially available in three different forms – namely, high-acyl, low-acyl and low-acyl clarified.

Gellan gum was previously evaluated by the Committee at its thirty-seventh meeting, at which an ADI “not specified” was established ([Annex 1](#), reference 95). This ADI “not specified” was based on the absence of adverse effects in toxicological studies in mice, rats, dogs and prepubertal rhesus monkeys and in a limited study on tolerance of gellan gum in humans. The Committee pointed out that the potential laxative effect of gellan gum at high dietary exposures should be taken into account when gellan gum is used as a food additive ([Annex 1](#), reference 95).

Gellan gum was evaluated by the Committee at its forty-ninth and seventy-ninth meetings for revision of specifications only ([Annex 1](#), references 124 and 222). At the seventy-ninth meeting, the Committee evaluated a request to include ethanol as an additional extraction solvent during the processing of gellan gum. The Committee at that meeting included ethanol in the specifications monograph and established a numerical limit of 50 mg/kg for residual ethanol ([Annex 1](#), reference 222).

At the present meeting, the Committee evaluated gellan gum for use in formulas for special medical purposes for infants (GSFA food category 13.1.3; referred to as “FSMPs” below) and re-evaluated the limit for residual ethanol in the specifications of gellan gum, at the request of the Fiftieth Session of CCFA [1]. Although the request from CCFA included the use of gellan gum in infant formula (GSFA food category 13.1.1) and follow-up formula (GSFA food category 13.1.2), only data supporting the use of gellan gum in FSMPs were received. Therefore, the Committee did not evaluate the use of gellan gum in infant formula or follow-up formula.

The low-acyl clarified form of gellan gum would be added directly to ready-to-feed FSMPs or would be used as a component of concentrated liquid fortification products⁵ formulated with hydrolysed protein and/or amino acids (for addition to human milk or infant formula). According to the sponsor, these liquid fortification products also belong to food category 13.1.3. Gellan gum would be used to increase thickness and maintain homogeneity for better delivery of nutrients to the infant. It would also be used as a component of a stabilizer system, which contains octenyl succinic anhydride–modified corn starch (starch sodium octenyl succinate) (INS 1450). The target gellan gum concentration in the fed products (FSMPs, fortified human milk or fortified infant formula) is approximately 40 mg/L. Owing to manufacturing variability, the maximum gellan gum concentration requested is 50 mg/L.

At the present meeting, the Committee considered the submitted data, including new unpublished and published studies. A comprehensive literature search on gellan gum in PubMed did not identify any additional relevant published studies on biochemical or toxicological aspects. Studies from the previously published monograph, new studies that had become available since the thirty-seventh meeting and older studies not previously reviewed by the Committee are described below.

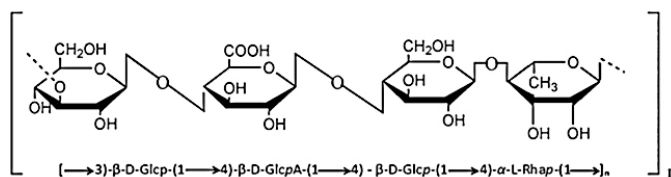
Chemical and technical considerations

Gellan gum is a high-molecular-weight (>500 000 Da) anionic polysaccharide that is produced by a controlled pure culture fermentation of the non-pathogenic Gram-negative bacterium *Pseudomonas elodea* (reclassified as *Sphingomonas elodea*) in the presence of a carbon source, a nitrogen source and inorganic salts. The fermentation broth is pasteurized to kill viable cells, and the gellan gum is recovered via precipitation with food-grade isopropanol or ethanol to obtain the high-acyl form (native gellan gum). Controlled treatment with hot alkali prior to alcohol precipitation results in deacylation and yields gellan gum with varying degrees of acylation, including the low-acyl form. Low-acyl gellan gum can be further filtered to obtain low-acyl clarified gellan gum. The gelling properties of the articles of commerce are controlled by the addition of metal ions such as sodium, potassium and calcium to neutralize the glucuronic acid. By-products of fermentation include polyhydroxybutyrate, enzymes and viable cells of the production organism, which are removed and/or inactivated during processing. The resulting gellan gum is separated, dried and milled.

In its native form, gellan gum is linear; it is composed of β -D-glucopyranosyl, β -D-glucuronopyranosyl and L-rhamnopyranosyl units in molar

⁵ Nutritional supplements designed to increase the total energy, protein and micronutrient delivery to preterm infants [2].

Fig. 3

Chemical structure of gellan gum backbone

ratios of 2:1:1 (Fig. 3). Native gellan gum also contains an acetyl and a glyceryl group bound to the glucose adjacent to the glucuronic acid residues.

Different types of gellan gum were used in the toxicological studies evaluated at the thirty-seventh meeting (Annex 1, reference 95) and in the new studies available for the current meeting. The gellan gum used in the acute toxicity studies [3, 4], the 13-week oral rat study [5] and the genotoxicity studies by Robertson and coworkers [6–9] was the low-acyl form, with greater than 95% polysaccharide content. The gellan gum samples used in the 52-week study in dogs [10], the long-term studies in mice and rats [11, 12], the genotoxicity study by Ivett [13], the special studies by Gordon [14, 15] and the studies in human adults [16, 17] were a blend of five products containing 58.5% polysaccharide (no further information available) with varying degrees of acylation. Low-acyl clarified gellan gum was used in the study in neonatal pigs [18], the clinical trials in infants [19–22] and the commercial products on which the post-marketing surveillance data were available [23–25]. The specific purity of the batches used was not provided. Based on the certificates of analyses of three representative batches submitted, these products are expected to contain greater than 94% polysaccharide. Characterization information on the gellan gum used in the other studies described below was not available.

Biochemical aspects

At its thirty-seventh meeting (Annex 1, reference 95), the Committee concluded, on the basis of rat studies with radiolabelled gellan gum, that gellan gum is poorly absorbed and primarily excreted in the faeces following oral administration [26].

For the present meeting, data from short-term studies in rats and human volunteer studies were submitted. A study in human volunteers provided no evidence of gellan gum absorption [27]. No statistically significant increases in short-chain fatty acid production were reported in animals or humans following gellan gum exposure, suggesting limited microbial degradation of gellan gum in the gastrointestinal tract. Increases were observed in faecal weights and water content, indicating that gellan gum may be a faecal bulking agent [16, 27–30].

In rats, reduced gastrointestinal transit times were reported after exposure to gellan gum [29–31], whereas in a study in human volunteers, variable effects on gastrointestinal transit times were observed [27].

Toxicological studies

At the thirty-seventh meeting ([Annex 1](#), reference 95), the Committee noted that gellan gum exhibited low acute oral toxicity, with an LD₅₀ value greater than 5000 mg/kg bw in rats [4]. Gellan gum did not cause adverse effects in a 90-day study in rats at doses up to 60 000 mg/kg feed (equivalent to 6000 mg/kg bw per day), a 52-week study in dogs at doses up to 60 000 mg/kg feed (equivalent to 1500 mg/kg bw per day assuming dry laboratory chow diet) and a 28-day study in prepubertal rhesus monkeys at doses up to 3000 mg/kg bw per day via gavage [5, 10, 32].

For the current meeting, an additional series of short-term studies in rats was available, focusing mainly on the gastrointestinal system [28–31, 33]. In all these studies, the animals were given gellan gum at a dietary concentration equivalent to 5000 mg/kg bw per day for 4 weeks. In the study by Tetsuguchi et al. [29], slight morphological changes in the intestinal mucosa were observed microscopically. The variations in the gastrointestinal mucosa were considered by the authors to be related to the gellan gum-induced increase in the viscosity of the intestinal contents, rather than a direct effect of gellan gum, and were not considered to be adverse, and the Committee agreed with this conclusion.

In summary, no adverse effects were reported in any of the short-term studies.

Two additional studies were conducted to specifically assess the potential effects of gellan gum on the gut epithelium and on mineral retention, respectively, using dietary concentrations equivalent to up to 5000 mg/kg bw per day [14, 15]. No adverse effects on intestinal morphology were reported after exposure of rats for 25 days [14]. Gellan gum did not affect growth or mineral retention after exposure of rats for 8 weeks [15].

Both available long-term toxicity and carcinogenicity studies were previously reviewed by the Committee at its thirty-seventh meeting ([Annex 1](#), reference 95). No treatment-related adverse effects or histopathological changes were reported following administration of gellan gum at dietary concentrations up to 30 000 mg/kg feed (equivalent to 4500 mg/kg bw per day) in mice for up to 98 weeks or 50 000 mg/kg feed (equivalent to 2500 mg/kg bw per day) in rats for 104 weeks [11, 12].

The Committee previously evaluated three *in vitro* genotoxicity studies on gellan gum, including a bacterial reverse mutation assay, a DNA repair assay (unscheduled DNA synthesis assay) and a gene mutation assay [6, 7, 9]. These studies all showed no evidence of genotoxicity.

Two additional genotoxicity studies were available for the present evaluation. In an alkaline elution assay, gellan gum was found to react with diaminobenzoic acid, forming a fluorescent product that interfered with DNA measurements. The authors therefore concluded that the assay was not valid [8], and the Committee agreed with this conclusion. Gellan gum gave negative results in an *in vivo* micronucleus assay [13]. However, the Committee noted that this result is not unexpected, given the poor absorption of gellan gum.

Considering the results of all available genotoxicity studies as well as the chemical structure of gellan gum, the Committee concluded that there is no concern for genotoxicity.

No adverse effects were reported in the reproductive and developmental toxicity studies in rats that were evaluated at the previous meeting [34, 35].

For the current meeting, the results of the *in utero* phase [36] of the long-term toxicity and carcinogenicity study in rats [12] were available. Treatment of the animals with gellan gum started 63 days prior to mating and was continued throughout mating, gestation and lactation. The NOAEL for parental, reproductive and offspring toxicity was 50 000 mg/kg feed (equal to 3520 mg/kg bw per day), the highest dose tested.

Special study in neonatal pigs

To assess the safety of gellan gum specifically as a component of infant formula, a study in neonatal pigs was submitted [18]. These pigs were fed milk replacer with gellan gum (low-acyl clarified product) at a concentration of 0, 41 or 205 mg/L (equal to 0, 19 and 100 mg/kg bw per day for males and 0, 20 and 100 mg/kg bw per day for females, respectively). The neonatal pigs were fed the gellan gum-containing milk replacer 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 or (fortified) human milk may be provided as the sole source of nutrition. The aim of this study was to investigate potential effects of gellan gum on growth and development, with emphasis on the gastrointestinal tract and immune system. No gross or microscopic changes were reported in the small or large intestine of the neonatal pigs. The incidence of pelvic dilatation in the kidneys (hydronephrosis) was higher than the background incidence in historical controls. However, as the severity was mild and there were no microscopic correlates, the Committee considered these findings in the kidney to be of no toxicological relevance. Histopathological examination of the non-glandular stomach revealed variable acute inflammation, hyperkeratosis and/or erosion in all groups of animals, including concurrent controls. The author considered the non-glandular stomach lesions likely to be incidental, as no dose-response relationship was observed and as the stomach lesions reported

were recognized as common observations in pigs. The Committee agreed with this conclusion.

The NOAEL for gellan gum was 205 mg/L (equal to 100 mg/kg bw per day), the highest dose tested [18].

Observations in humans

Results from a previously evaluated, limited study on tolerance of gellan gum in adult humans indicated that daily oral doses of up to 200 mg/kg bw administered over a 23-day period did not elicit any adverse reaction, although faecal bulking effects were observed in most subjects. In two males, an increase in the percentage of eosinophils was observed, and the number of eosinophils in one of the subjects was reported to fall outside the normal range [16, 27]. Therefore, a follow-up study was performed to exclude possible sensitizing effects of gellan gum in 20 human volunteers, among whom were the two male volunteers who had elevated eosinophils in the previous study [17, 37]. No allergic reactions were observed among the subjects during or following gellan gum dietary supplementation, and no changes were observed in haematological parameters. Based on the results, the Committee concluded that there are no indications that gellan gum is sensitizing [17, 37].

Four paediatric clinical studies were conducted in preterm infants (gestational age <33 weeks, birth weight <2000 g) with human milk fortification (HMF) products containing gellan gum (low-acyl clarified form) for consumption by preterm and/or very low birth weight infants [19–22]. The products evaluated in these studies included powdered HMF products, acidified liquid HMF (AL-HMF) products (sterilized by acidification) and non-acidified liquid HMF (NAL-HMF) products (sterilized by heat treatment). Gellan gum was an ingredient of the NAL-HMF products. For the four studies taken together, 214 infants received human milk with NAL-HMF products containing gellan gum, and 226 infants received human milk with HMF products without gellan gum. The infants were enterally fed with human milk fortified with HMF products for 29–40 days. The gellan gum concentration in the fortified human milk was approximately 40 mg/L, and the dietary exposure ranged from approximately 3 to 6 mg/kg bw per day. No adverse effects on growth, haematological or biochemical parameters or clinical outcomes were reported with NAL-HMF products containing gellan gum when compared with the other HMF products tested, except for an increase in reticulocyte count in a pilot study by Kumar et al. [21]. The authors indicated that this could possibly be explained by the ferrous sulfate that was given to the NAL-HMF group because of the lower iron content of NAL-HMF products compared with AL-HMF products, but noted that this finding would need confirmation in larger studies. The Committee noted that the HMF products tested differed in several ways (protein content, protein type [hydrolysed vs intact], powder vs

liquid, acidified vs non-acidified, different food additives), so these studies do not provide information specifically about gellan gum. However, these studies did show that the tested NAL-HMF products containing gellan gum were generally well tolerated.

Post-marketing surveillance data over a 2.5-year period showed that the use of gellan gum (low-acyl clarified form) was well tolerated when administered to preterm infants through its use in an HMF product resulting in concentrations in human milk of approximately 40 mg/L [23–25].

Assessment of dietary exposure

At the current meeting, the Committee estimated the dietary exposure to gellan gum from its use in FSMPs and in concentrated liquid fortification products for addition to human milk or infant formula, as proposed by the sponsor. The requested maximum concentration of gellan gum in the fed products (FSMPs, fortified human milk or fortified infant formula) is 50 mg/L. Dietary exposure to gellan gum was assessed using consumption data for infant formula based on enteral feeding volumes of preterm infants, WHO-recommended consumption levels, consumption levels based on estimated energy requirements and actual reported consumption levels.

Based on the different consumption levels, the dietary exposure to gellan gum at the requested maximum concentration of 50 mg/L in fed products was estimated to range from 3.0 to 13 mg/kg bw per day. The dietary exposure of 13 mg/kg bw per day was based on a high level of consumption of infant formula of 260 mL/kg bw per day as derived by the Scientific Committee of EFSA [38]. This high consumption level also covers the potential high consumption of preterm infants on formula feeding [38].

The Committee noted that no dietary exposure assessment was performed for gellan gum for any food uses at the previous meeting ([Annex 1](#), reference 95).

Evaluation

The Committee previously established an ADI “not specified” for gellan gum ([Annex 1](#), reference 95). The ADI “not specified” was based on the absence of toxicity in animal studies, including long-term studies in mice and rats and a 52-week study in dogs in which animals were fed gellan gum at doses up to, respectively, 4500 mg/kg bw per day, 2500 mg/kg bw per day and 1500 mg/kg bw per day.

Several additional *in vitro* studies, animal studies and human data related to the safety of gellan gum have become available since the Committee’s last evaluation. Results confirm the absence of any adverse effects arising from exposure to gellan gum. Therefore, the Committee retained the previously established ADI “not specified” for gellan gum.

ADIs established on the basis of the usually provided toxicology data are not applicable to infants up to the age of 12 weeks. The previously evaluated toxicity studies did not include direct oral administration to neonatal animals and thus did not address safety for the young infant age group. At the present meeting, a 21-day neonatal pig study using low-acyl clarified gellan gum, which modelled the 0- to 12-week period of development in human infants, was evaluated. The NOAEL was 100 mg/kg bw per day, the highest dose tested. Based on this NOAEL and the high estimate of dietary exposure of infants to gellan gum of 13 mg/kg bw per day (based on the requested maximum concentration of gellan gum of 50 mg/L and the high level of consumption of infant formula of 260 mL/kg bw per day), an MOE of 7.7 was calculated.

To interpret an MOE related to exposure in infants, the Committee has previously established several considerations that need to be addressed ([Annex 1](#), reference 220). If these considerations are met, MOEs in the region of 1–10 may indicate low risk for the health of 0- to 12-week-old infants exposed to the food additive through infant formula ([Annex 1](#), reference 220). The considerations relevant for the current evaluation of gellan gum for use in FSMPs and liquid fortification products for addition to human milk or infant formula are as follows:

- No adverse effects were observed in any of the studies available, indicating that the toxicity of gellan gum is low.
- The NOAEL was the highest dose tested in a study in neonatal pigs, which are considered a relevant animal model for human infants.
- Clinical studies in preterm infants support the tolerability of HMF products containing gellan gum resulting in concentrations of gellan gum in human milk up to approximately 40 mg/L.
- Post-marketing surveillance data over a 2.5-year period showed that the use of gellan gum was well tolerated when administered to preterm infants through its use in an HMF product resulting in concentrations in human milk of approximately 40 mg/L.
- The dietary exposure estimate was based on the requested maximum concentration of gellan gum of 50 mg/L.
- A high level of consumption of infant formula (260 mL/kg bw per day) was used to assess the dietary exposure.

Based on these considerations, the Committee concluded that the MOE of 7.7 calculated for the use of gellan gum in FSMPs and liquid fortification products for addition to human milk or infant formula at a maximum level of 50 mg/L in the fed product indicates low risk for the health of infants, including preterm infants, and that its proposed use is therefore of no safety concern. This conclusion applies only to the use of low-acyl clarified gellan gum. The

Committee recognizes that there is variability in medical conditions among infants requiring these products and that these infants would normally be under medical supervision.

A consolidated toxicological and dietary exposure monograph was prepared.

The Committee discussed the request to revise the limits on residual ethanol. Based on the data submitted, the Committee concluded that the use of ethanol in the manufacturing of gellan gum is not a safety concern when used in accordance with good manufacturing practice. The specification for ethanol was removed, and the existing specifications for gellan gum were revised. The specifications were made tentative, pending submission of new methods for characterizing the three forms of gellan gum in commerce by 2021.

A Chemical and Technical Assessment was prepared.

Recommendations

The specifications were made tentative, pending submission of new methods for characterizing the three forms of gellan gum in commerce by 2021. Specific information required is as follows:

- A method to differentiate the three commercial forms of gellan gum – i.e. high-acyl, low-acyl and low-acyl clarified.
- A method to determine the degree of acylation.
- Validation data for the above methods, including detailed description of the sample preparation.
- Data from five non-consecutive commercial batches of material using the proposed validated methods for all three forms of gellan gum.

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3.1.5 Potassium polyaspartate

Explanation

Potassium polyaspartate (INS 456) is a food additive intended to be used as a stabilizer to prevent tartrate crystal precipitation in wine at a proposed maximum use level of 300 mg/L. Potassium polyaspartate is produced from L-aspartic acid and potassium hydroxide.

Potassium polyaspartate has not previously been evaluated by the Committee. L-Aspartic acid is a component of the sweetener aspartame, which was evaluated by the Committee at its nineteenth, twentieth, twenty-first, twenty-third, twenty-fourth, twenty-fifth and eighty-second (specifications only) meetings ([Annex 1](#), references 38, 41, 44, 50, 53, 56 and 230), and the use of

L-aspartic acid as a flavouring agent was evaluated by the Committee at its sixty-third meeting ([Annex 1](#), reference 173). Potassium hydroxide is a food additive (INS 525; CAS No. 1310-58-3) that was evaluated by the Committee at its ninth meeting ([Annex 1](#), reference 11).

Potassium polyaspartate was placed on the agenda of the present meeting at the request of the Fiftieth Session of CCFA [1] for an assessment of its safety, dietary exposure and specifications. The sponsor submitted unpublished toxicological studies and published papers. Two additional relevant publications were identified in a literature search. The Committee also considered the components of potassium polyaspartate using previous JECFA evaluations and other reviews. The sponsor provided details of typical and maximum use levels in wine and a dietary exposure assessment for Europe. Published estimates of dietary exposure noted by the sponsor were also reviewed. A literature search did not identify any additional estimates of dietary exposure.

Chemical and technical considerations

Potassium polyaspartate is produced from L-aspartic acid in a two-step process. During the first step, heating of solid L-aspartic acid leads to solid-phase polycondensation and production of polysuccinimide. Racemization occurs during this step [2], leading to the occurrence of both D- and L-aspartic acid in the final product. The water-insoluble polysuccinimide obtained is subsequently treated with aqueous potassium hydroxide under controlled conditions, which leads to hydrolysis, opening of the succinimide rings and production of the water-soluble potassium salt. The product contains approximately 70% β -peptide bonds and 30% α -peptide bonds. The final spray-dried potassium polyaspartate is a low-molecular-weight, polydisperse polymer with a weight-average molecular weight of approximately 5000 Da and a number-average molecular weight of about 1000 Da. Up to 20% has a molecular weight of less than 1000 Da.

Biochemical aspects

There are no *in vivo* data on the absorption of potassium polyaspartate.

In vitro data on Caco-2 monolayers that were used to simulate gastrointestinal absorption [3, 4] suggest that the systemic bioavailability of potassium polyaspartate is low. Other *in vitro* data obtained with pepsin and pancreatin to simulate gastrointestinal digestion [3] suggest that potassium polyaspartate would not be cleaved in the stomach and the intestine. However, potassium polyaspartate could be digested by microbiota occurring in the human intestine. The Committee noted the absence of information on the extent of fermentation of polyaspartate.

Toxicological studies

No information on the acute toxicity of potassium polyaspartate was available.

A dose range-finding study in rats given potassium polyaspartate by oral gavage at a dose of 0, 60, 125, 250, 500 or 1000 mg/kg bw per day for 14 days showed no treatment-related adverse effects [5, 6].

In a 90-day toxicity study, rats were given potassium polyaspartate by oral gavage at a dose of 0, 250, 500 or 1000 mg/kg bw per day. No treatment-related adverse effects were observed. The NOAEL was 1000 mg/kg bw per day, the highest dose tested [6, 7].

No long-term toxicity or carcinogenicity studies were available.

A bacterial reverse mutation assay and an in vitro micronucleus assay in human lymphocytes gave negative results [6, 8, 9]. The Committee concluded that there is no concern with respect to the genotoxicity of potassium polyaspartate.

No specific studies on reproductive and developmental toxicity, neurotoxicity or immunotoxicity were available. However, the 90-day study described above included additional parameters that provide information on some of these end-points. No effects on the estrous cycle or on weights and histopathology of testes, epididymides, seminal vesicles, uterus or ovaries were observed. There were no signs of neurological dysfunction investigated using a functional observational battery approach. No treatment-related effects indicating an immunotoxic or immunomodulatory potential were observed. In addition, histopathological investigations performed on thyroid and parathyroid and blood concentrations of triiodothyronine (T_3), thyroxine (T_4) and thyroid stimulating hormone (TSH) measured at termination of the treatment found no treatment-related effects that indicated disturbance of thyroid function [6, 7].

Results from an in vitro study in which the human promyelocytic cell line THP-1 was used as a surrogate for monocytes did not provide any indication of an immune response as indicated by CD86 expression and interleukin 8 release [3].

Observations in humans

No information was available.

Studies on L- and D-aspartic acid and potassium

Because the aspartic acid incorporated in the polyaspartate backbone is in an L- and D- configuration, the Committee considered L- and D-aspartic acid resulting from possible breakdown of potassium polyaspartate, as well as potassium.

L-Aspartic acid

L-Aspartic acid is a non-essential amino acid that occurs in food. It is also a component of the intense sweetener aspartame. Because L-aspartic acid results

from the hydrolysis of aspartame, the toxicity of and dietary exposure to L-aspartic acid were considered by the Committee in the course of its evaluations of the use of aspartame. The Committee concluded that L-aspartic acid generated from aspartame was not a safety concern at current dietary exposure to aspartame ([Annex 1](#), reference 54).

When the use of L-aspartic acid as a flavouring agent was evaluated by the Committee at its sixty-third meeting, the Committee concluded that there was no safety concern at current dietary exposures when used as a flavouring agent ([Annex 1](#), reference 174).

D-Aspartic acid

D-Aspartic acid is an endogenous amino acid that is involved in the development of the nervous system, plays a role in the neuroendocrine system, including hormone synthesis, has neuronal activities and is implicated in male fertility [10–23]. D-Aspartic acid is present in the human brain and accumulates with age in the central nervous system white matter, but not in grey matter [24, 25].

In a systematic review of 23 animal studies, three of which involved oral exposure of rats, and four human studies, the authors concluded that exogenous D-aspartic acid enhances testosterone levels in male animals at oral doses equivalent to around 130 mg/kg bw per day, whereas studies in humans, in which daily doses ranging from 36 to 70 mg/kg bw per day were consumed as dietary supplements, yielded inconsistent results. The authors noted that the inconsistent results obtained in these human trials could be due to limitations of the study designs, such as short-term supplement duration (12–28 days) and small sample sizes ($N = 10–23$ in the supplemented groups) [26]. The Committee agreed with this conclusion and noted that no NOAELs could be identified from the oral rat studies, as only single doses were tested.

There is experimental evidence for an L-isomer-selective transport of aspartic acid at the blood–brain barrier in the rat, whereby L-aspartic acid, but not D-aspartic acid, undergoes efflux transport from the brain to the blood; in contrast, the uptake of aspartic acid in brain parenchymal cells is not stereospecific [27, 28]. However, the Committee noted that administration of D-aspartic acid to rats in drinking-water at a dose of 50 mg/kg bw per day for 28 days increased its levels in both liver and blood serum about 5-fold and in kidney homogenates 8-fold, but did not increase the D-aspartate level in brain homogenates [29]. The Committee also noted that while the study did not meet current standards applicable for repeated-dose 28-day oral toxicity studies in rats (OECD Test Guideline 407), no signs of general toxicity were detected, and histopathological evaluation of renal and hepatic tissues did not reveal any treatment-related pathological alterations.

The Committee further noted that free D-aspartic acid can be metabolized by D-amino acid oxidase, which is expressed in brain, spinal cord, liver, renal proximal tubule cells and the proximal and middle small intestine of mice and humans [30].

There are no longer-term (>1 month) oral toxicity studies on D-aspartic acid and no toxicity studies on racemic mixtures of D- and L-aspartic acid.

Potassium

Potassium was evaluated by the Committee in the course of the evaluation of potassium hydroxide as a food additive at its ninth meeting ([Annex 1](#), reference 11). The result of the evaluation was an ADI “not limited”⁶ for potassium hydroxide.

Serum levels of potassium usually rise only moderately in response to potassium intake, even in the case of a short-term (2–24 weeks) high potassium intake of 1755 mg/day, which resulted in an increase in potassium serum levels by only 0.17 mmol/L (6.6 mg/L) [31].

Assessment of dietary exposure

A dietary exposure assessment for potassium polyaspartate was undertaken for the first time by the present Committee. The assessment was based on typical use levels in wine of 100–200 mg/L and a maximum proposed use level of 300 mg/L.

Estimated dietary exposures reviewed were those submitted by the sponsor based on EFSA’s FAIM, an EFSA assessment based on the Comprehensive European Food Consumption Database [32] and national dietary survey data for Australia and New Zealand [33]. The Committee also calculated national estimates of dietary exposure based on food consumption data in CIFOOCs for Brazil, China and the USA.

A summary of the national dietary exposure estimates is shown in [Table 3](#).

The estimates of dietary exposure to potassium polyaspartate based on the maximum use level are overestimates; instead, the exposures based on typical use levels provide better estimates of chronic dietary exposures. Mean estimates of dietary exposure based on typical use levels are up to 0.7 mg/kg bw per day, and high exposures are up to 1.6 mg/kg bw per day.

L-Aspartic acid used in the manufacture of potassium polyaspartate also occurs naturally in food and can be consumed via dietary supplements and food additives such as aspartame. The Committee estimated that the dietary exposure for each of L- and D-aspartic acid is up to 0.8 mg/kg bw per day from the typical use of potassium polyaspartate in wine. This represents 50% of the total aspartic acid exposure (for both L- and D-aspartic acid) of 1.6 mg/kg bw per

⁶ Now called ADI “not specified”.

Table 3

Range of estimated dietary exposures^a to potassium polyaspartate at typical and maximum food additive use levels

Population group	Estimated dietary exposure (mg/kg bw per day)			
	Typical use level: 100–200 mg/L		Maximum use level: 300 mg/L	
	Mean	High ^b	Mean	High ^b
Children	0–0.06	0–0.14	0–0.09	0–0.22
Adults	0.01–0.68	0–1.52	0.02–1.02	0–2.28
General population	0.09–0.70	0.18–1.58	0.26–1.05	0.54–2.37

bw: body weight

^a Includes estimates for Europe and national estimates submitted to and calculated by the Committee.^b High exposure is the 90th percentile for all estimates other than Europe, for which high exposure is the 95th percentile.

day due to racemization and assumes that potassium polyaspartate is completely fermented in the colon and that the products of the fermentation are absorbed and bioavailable.

Estimated dietary exposure to L-aspartic acid from the food additive use is around 1% of a mean population dietary exposure of 108 mg/kg bw per day (6.5 g/day) for total aspartic acid from the diet (natural and supplemental sources) [34] and less than 1% of a high dietary exposure of 200 mg/kg bw per day (12.0 g/day) for total aspartic acid. The Committee concluded that the amount of additional L-aspartic acid in the diet from potassium polyaspartate is negligible and would be within normal daily variation in dietary exposures.

Dietary exposures to D-aspartic acid from six foods known to contain it (milk, cheese, yoghurt, beer, wine, juice) were estimated. Dietary exposures from the individual foods ranged between 0.001 and 0.07 mg/kg bw per day. The Committee was aware that this is an incomplete list of foods and also noted that food processing (e.g. heat treatment of protein, fermentation) will result in partial conversion of L-aspartic acid to D-aspartic acid for a range of other foods. Therefore, total dietary exposure to D-aspartic acid would be higher than estimated here.

The Committee considered the additional dietary exposure to potassium in the diet from use of the food additive and estimated a dietary exposure to potassium of about 45 mg/day for high consumers of wine. This is well below usual dietary exposures of between 2000 and 3000 mg/day, and the Committee concluded that the additional dietary exposure to potassium from use of the food additive in wine would be within normal daily variation.

Evaluation

In vitro data suggest that the systemic bioavailability of potassium polyaspartate is low and that it would not be cleaved in the stomach or the intestine. The NOAEL

in a 90-day rat study on potassium polyaspartate was 1000 mg/kg bw per day, the highest dose tested. There was no concern for genotoxicity.

Potassium has been evaluated by the Committee in the course of its evaluation of potassium hydroxide ([Annex 1](#), reference 11), and the result of the evaluation was an ADI “not limited”. Exposure to potassium that results from the use of potassium polyaspartate in wine would be within normal daily variation of background potassium exposure from the diet.

The Committee noted that no information on potential microbial fermentation in the human colon is available, but should that occur, there would be potential exposure to L- and D-aspartic acid. L-Aspartic acid is a normal constituent of dietary protein, and systemic exposure to L-aspartic acid from the diet is much higher than potential exposure from the use of potassium polyaspartate in wine.

There are no relevant toxicological data on D-aspartic acid. In three studies, rats exposed to around 130 mg/kg bw per day showed effects on sex hormone levels. However, NOAELs have not been identified in these studies due to the use of single doses. The Committee noted that there is an MOE of more than 100-fold between the potential human exposure to D-aspartic acid of up to 0.8 mg/kg bw per day and the effect level of 130 mg/kg bw per day.

The estimated dietary exposure to D-aspartic acid from typical use of potassium polyaspartate in wine (up to 0.8 mg/kg bw per day) would be expected to be lower than the exposure from non-added sources in the diet. The Committee noted that it had limited data on concentrations of D-aspartic acid in food, but that food processing (e.g. heat treatment of protein, fermentation) will result in partial conversion of L-aspartic acid to D-aspartic acid.

The Committee concluded that the use of potassium polyaspartate in wine at the maximum proposed use level of 300 mg/L is not of safety concern.

A toxicological and dietary exposure monograph was prepared.

New specifications for potassium polyaspartate were prepared.

A Chemical and Technical Assessment was prepared.

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3.1.6 Rosemary extract

Explanation

Rosemary extract (INS 392) is an antioxidant food additive obtained from ground dried leaves of *Rosmarinus officinalis*. The antioxidant properties 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.

The Committee previously evaluated rosemary extract at its eighty-second meeting ([Annex 1](#), reference 230). At that meeting, the Committee established a temporary ADI of 0–0.3 mg/kg bw for rosemary extract, expressed as carnosic acid plus carnosol. This ADI was based on a NOAEL of 64 mg/kg bw per day, the highest dose tested in a short-term toxicity study in rats. An uncertainty factor of 200 was used, which includes an uncertainty factor of 100 and an additional uncertainty factor of 2 to account for the temporary designation of the ADI, 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 the rosemary extract that met the specifications prepared at the eighty-second meeting.

Rosemary extract was placed on the agenda of the present meeting at the request of the Fiftieth Session of CCFA [1] for an assessment of its safety, dietary exposure and specifications, including studies to elucidate its potential developmental and reproductive toxicity, information to validate the method of determination of residual solvents and data on typical use levels in food. A study on the reproductive and developmental toxicity of an acetone-based rosemary extract was submitted by the sponsors. In addition, a literature search identified five relevant studies published after the eighty-second meeting of JECFA. The Committee reviewed the data on typical use levels in food that were provided for the present meeting. In addition, updated dietary exposure assessments based on maximum permitted levels were available for review, as were assessments based on typical use levels. A literature search was also undertaken; however, it did not identify any further information on typical use levels or estimates of dietary exposure to rosemary extract.

Chemical and technical considerations

No new manufacturing information was submitted. The Committee received validation data and information on the method for determination of ethanol and acetone used during the manufacturing of rosemary extract.

Biochemical aspects

In a pharmacokinetics study that investigated an ethanol-based extract of dried leaves of rosemary [2], rats were administered rosemary extract at a dose of 240,

820 or 2450 mg/kg bw by oral gavage. Plasma concentrations of carnosic acid and carnosol were determined up to 24 hours after administration. The time at which the maximum concentration (C_{\max}) was reached (T_{\max}) was approximately 0.5 hour. The C_{\max} and area under the plasma concentration versus time curve from time 0 to infinity ($AUC_{0-\infty}$) values showed reasonably good agreement, with a proportional increase with dose. An apparent double-peak phenomenon in the plasma concentration versus time curves, suggesting redistribution and enterohepatic recirculation, was also observed [2]. The Committee noted inconsistencies in C_{\max} and T_{\max} values between this study and two previously evaluated studies [3, 4].

A study by Seow & Lau [5] using a luciferase reporter gene assay with human (hPXR), mouse (mPXR) and rat (rPXR) pregnane X receptors indicated that carnosol is an activator of all three receptors, whereas carnosic acid is a potent agonist of both hPXR and mPXR, but not rPXR. These new findings provide insight on the molecular basis for the pregnane X receptor-mediated induction of expression of phase 1 and phase 2 enzymes of xenobiotic metabolism and membrane transport proteins.

An *in vitro* study by Ercan & El [6] showed that a rosemary water extract with 18.7% carnosic acid was a potent inhibitor of pancreatic lipase.

Toxicological studies

A new OECD-compliant (Test Guideline 421) reproductive/developmental toxicity screening study in rats using an acetone extract of rosemary with a high content of carnosic acid was available [7]. Rats were administered rosemary extract in the diet at initial concentrations of 0, 2100, 3600 and 5000 mg/kg feed, which were later reduced in females from gestation day 20 to 0, 1050, 1800 and 2500 mg/kg feed (equal to 0, 130, 219 and 316 mg/kg bw per day for males and 0, 167, 276 and 401 mg/kg bw per day for females, respectively). No adverse effects were observed in parental males or females or in reproductive parameters. Gestation length, litter size and pup body weight on postnatal day 1 and pup survival and body weight gain until postnatal day 13 (termination) were not affected by treatment. A clear dose-related reduction in total- T_4 serum levels in male and female pups was observed on postnatal day 13. Histopathological examination of the thyroid gland (one male and one female pup per litter) showed no abnormality [7]. The Committee noted the high variability in the thyroid hormone measurements in the pups.

A NOAEL of 5000 mg/kg feed (equal to 316 mg/kg bw per day), the highest dose tested, was identified for reproductive and parental toxicity. The Committee noted that it was unclear whether the treatment-related effects on thyroid hormone levels in pups were adverse, and therefore a NOAEL for offspring toxicity could not be identified. The study also did not provide adequate

evidence for the absence of developmental toxicity, given that no fetuses were examined.

One toxicological study on carnosic acid was identified in the literature search. Liu et al. [8] tested carnosic acid in an in vitro screening assay for embryotoxic potential using mouse embryonic stem cells. The embryonic stem cell test is an extensively used screening assay for developmental toxicity that has been validated by the European Union Reference Laboratory for alternatives to animal testing [9]. Studies on the predictivity of the embryonic stem cell assay indicated a significant false-positive rate (approximately 40%), but a very low false-negative rate (approximately 7%) [9]. According to the results from this in vitro assay, carnosic acid is weakly embryotoxic [8].

Observations in humans

A small-scale clinical study (a randomized, double-blinded and placebo-controlled study) investigated the memory-enhancing effects of a combined ethanol extract of three plants, including *Rosmarinus officinalis*. No adverse effects of the combined ethanol extract following administration for 14 days were reported [10]. The Committee noted that this study does not contribute to the evaluation.

Assessment of dietary exposure

The Committee first evaluated dietary exposure to rosemary extract (expressed as carnosic acid plus carnosol) at its eighty-second meeting ([Annex 1](#), reference 230). At that time, the estimates were based on maximum permitted and proposed levels. The Committee at that meeting noted that the dietary exposure estimates for high consumers of 0.09–0.81 mg/kg bw per day may exceed the upper bound of the temporary ADI by up to 2.7-fold. Based on the conservative nature of the dietary exposure assessments, the Committee requested that data on typical use levels in foods be provided in order to refine the dietary exposure estimates.

At the current meeting, typical use levels of rosemary extract (expressed as carnosic acid plus carnosol) from Europe [11], Australia and New Zealand [12] were available to the Committee for review. Dietary exposure assessments (expressed as carnosic acid plus carnosol) were also available based on typical use levels. These included estimates for Europe based on typical use levels in Europe [11], estimates for Australia and New Zealand based on typical use levels for those countries [12] and an assessment for the USA (from the sponsors) based on concentrations that were between the range of typical use and maximum permitted levels from the European Union, Australia and New Zealand. Although estimates of dietary exposure were also provided based on maximum permitted levels, only estimates of dietary exposure based on typical use levels were used

by the Committee in the evaluation. In addition, only non-brand-loyal results for Europe were used for the purpose of the evaluation.

For children, mean estimates of dietary exposure ranged between <0.01 and 0.14 mg/kg bw per day; high-percentile exposures ranged between <0.01 and 0.30 mg/kg bw per day. For adults, mean estimates of dietary exposure ranged between <0.01 and 0.05 mg/kg bw per day; high-percentile exposures ranged between 0.01 and 0.12 mg/kg bw per day. Estimated dietary exposures based on typical use levels were less than half those estimated by the Committee at the eighty-second meeting, which were based on maximum permitted levels, at the upper ends of the ranges of both mean and high-percentile exposures. Depending on the country, the main contributors to dietary exposure were fine bakery wares, soups and broths, sauces and toppings (including mayonnaise and salad dressings) and processed meat products.

For the present meeting, estimates of dietary exposure from naturally occurring sources were available for Europe (rosemary and other herbs) [11] and Australia and New Zealand (rosemary only) [12]. Estimates included dietary exposures from naturally occurring sources only and in combination with added sources.

For naturally occurring sources only, estimated dietary exposures for children ranged between 0.0 and 0.34 mg/kg bw per day for mean exposures and between 0.0 and 1.66 mg/kg bw per day for high-percentile exposures. Estimated dietary exposures for adults ranged between 0.0 and 0.18 mg/kg bw per day for mean exposures and between 0.0 and 0.52 mg/kg bw per day for high-percentile exposures. When dietary exposures from naturally occurring sources are combined with dietary exposures from added sources at typical use levels, the estimated mean and high-percentile dietary exposures were up to 0.42 mg/kg bw per day for children and up to 0.16 mg/kg bw per day for adults (both estimates from Europe; mean naturally occurring dietary exposure added to mean food additive dietary exposure, eliminating the high dietary exposure of up to 0.52 mg/kg bw per day). The contribution from naturally occurring sources was <1–4% for Australia and New Zealand, based on the distribution of dietary exposures for individuals, and 65–93% for Europe, based on summing mean dietary exposures from added and natural sources.

Evaluation

The Committee concluded that the new studies provided evidence for the absence of reproductive toxicity, but not for the absence of developmental toxicity. The Committee retained the temporary ADI of 0–0.3 mg/kg bw, pending the submission of studies on the developmental toxicity of rosemary extract and studies to elucidate whether the effects noted on rodent pup thyroid hormone

levels can be replicated. The temporary ADI will be withdrawn if the requested studies are not submitted by the end of 2021.

Estimated mean and high-percentile dietary exposures to carnosic acid plus carnosol from use of rosemary extract as a food additive for all countries assessed based on typical use levels did not exceed the upper end of the temporary ADI of 0–0.3 mg/kg bw. The Committee noted that when dietary exposures from naturally occurring sources are combined with dietary exposures from added sources at typical use levels, the estimated dietary exposures for children were up to 0.42 mg/kg bw per day, which exceeds the ADI. The Committee noted that the temporary ADI is based on the highest dose tested in a short-term toxicity study in rats and that in the newly submitted reproductive/developmental toxicity screening study, no effects on reproductive toxicity or on parental animals were observed at 316 mg/kg bw per day, the highest dose tested. Therefore, the Committee does not consider the slight exceedance of the ADI to be a safety concern.

An addendum to the toxicological and dietary exposure monograph was prepared.

The Committee removed the specification for ethanol. The specifications monograph for rosemary extract was revised, and the tentative status was removed.

Recommendations

Studies on the developmental toxicity of rosemary extract and studies to elucidate whether the effects noted on pup thyroid hormone levels can be replicated were identified as research needs to complete the evaluation. The Committee requests that this information be provided by the end of 2021.

References

1. FAO/WHO. Report of the 50th Session of the Codex Committee on Food Additives, Xiamen, China, 26–30 March 2018. Rome, Italy: Food and Agriculture Organization of the United Nations; and Geneva, Switzerland: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2018 (REP18/FA).
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3.2 Revision of specifications

3.2.1 Citric and fatty acid esters of glycerol

CITREM was on the agenda of the current meeting at the request of the eighty-sixth meeting of JECFA to replace an obsolete packed column gas chromatographic method for the determination of total citric acid content ([Annex 1](#), reference 241). The Committee received a suitable validated replacement method, along with performance characteristics of the method and data on the total citric acid content in products currently available in commerce, determined using that method. The Committee included the new method in the specifications and deleted the previous method.

The Committee also considered the replacement of the method for glycerol to avoid the use of chloroform. A new HPLC method for the analysis of glycerol, supported by validation data, was provided and included in the revised specifications. The limit for glycerol was maintained.

Data on the use of additional neutralizing salts in CITREM manufacture were received and added to the specifications.

The lead limit for use of CITREM in infant formula was corrected to 0.5 mg/kg according to the previous evaluation.

The limit for sulfated ash was maintained for non-neutralized CITREM, and new limits were set for partially neutralized and for wholly neutralized CITREM.

Data on the sulfated ash levels and the content of minerals in neutralized CITREM products were provided. The Committee noted that although different neutralizing agents were used, this did not affect the current limit for sulfated ash.

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

3.2.2 **Metatartaric acid**

Metatartaric acid was on the agenda of the current meeting at the request of the Fiftieth Session of CCFA (7) to revise the specifications. The Committee, at its current meeting, received information on optical rotation, infrared identification, free tartaric acid content, degree of esterification and molecular weight distribution together with the analytical methods. The Committee revised the specifications for free tartaric acid, optical rotation, molecular weight and molecular weight distribution and included a specification for polydispersity index.

The specifications for metatartaric acid were revised, and the tentative status was removed. The Chemical and Technical Assessment was revised.

3.2.3 **Mannoproteins from yeast cell walls**

Yeast extracts containing mannoproteins was on the agenda of the current meeting at the request of the Fiftieth Session of CCFA (7) in order to complete the specifications related to the identity and purity of the product of commerce.

Given the additional compositional information received, the Committee revised the specifications monograph and noted that a change in the name of the food additive to “Mannoproteins from yeast cell walls” was appropriate. The Committee noted that all mannoproteins, regardless of the range of molecular weights, were included in the same specifications monograph and therefore that specifying a range of average molecular weight and a method for measuring it was not essential. Data were also received on metallic impurities. The Committee reviewed the information received and decided that only a limit for lead was required.

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



4. Flavouring agents

4.1 Specifications of identity and purity of flavouring agents

4.1.1 Revised specifications

The Committee received information in support of revision of the full specifications for nine flavouring agents that were on the agenda of the present meeting (JECFA Nos 141, 345, 547, 889, 893, 967, 979, 1029 and 1236).

The Committee revised specifications for methyl propionate (No. 141) and revised the specific gravity to 0.912–0.918 based on data from 20 lots of commercial product.

For ethyl oleate (No. 345), the Committee revised the assay minimum to not less than 75% ethyl oleate based on 29 lots of commercial product. Specifications for the secondary components were also established: ethyl linoleate (3.4–11.5%), ethyl palmitate (0.4–5.1%), ethyl stearate (0.5–2.5%), ethyl laurate (1–2%) and other fatty acid ethyl esters. The secondary components were not considered to pose a safety concern when No. 345 is used as a flavouring agent at current levels of use, as noted in [Annex 3](#).

The Committee revised specifications for alpha-methyl-beta-hydroxypropyl alpha-methyl-beta-mercaptopropyl sulfide (No. 547) based on data from flavouring agent currently in commerce and revised the refractive index to 1.512–1.522, the specific gravity to 1.040–1.050 and the assay minimum to 95%.

For vanillin (No. 889), the Committee reviewed data from 70 lots of commercial product and revised the melting point to 81–84 °C.

For ethyl vanillin (No. 893), the Committee reviewed data from 45 lots of commercial product and revised the melting point to 76–79 °C.

For 2,2,3-trimethylcyclopent-3-en-1-yl acetaldehyde (No. 967), the Committee reviewed data from three lots of commercial product and revised the assay minimum to 93%, with a secondary component of up to 2% of gamma-campholenic aldehyde. The secondary component was not considered to pose a safety concern when No. 967 is used as a flavouring agent at current levels of use, as noted in [Annex 3](#).

For alpha- and beta-cyclocitral (50:50 mixture) (No. 979), the Committee revised the specifications to include the CAS numbers for alpha-cyclocitral (CAS No. 432-24-6) and for the mixture of alpha- and beta-cyclocitral (CAS No. 52844-21-0). The Flavis and Council of Europe numbers for alpha- and beta-cyclocitral were also included. The refractive index range was revised to 1.4986–1.4991 based on information provided on the commercial product.

For sodium 2-(4-methoxyphenoxy)propanoate (No. 1029), the Committee revised the CAS number (CAS No. 150436-68-3) and Flavis number

(Flavis No. 08.127) to reflect the salt form. The melting point was revised to 184–190 °C based on information provided on the commercial product. Identifiers and synonyms associated with the free acid were removed.

Based on information provided on 60 lots of commercial product, the Committee revised the specifications for 2,2,6-trimethyl-6-vinyltetrahydropyran (No. 1236) by changing the minimum assay to 95%, the refractive index to 1.442–1.452 and the specific gravity to 0.863–0.873.

5. Future work and recommendations

Unsulphonated primary aromatic amines in food colours

The Committee requests analytical data on unsulphonated primary aromatic amines in the following synthetic food colours – Allura Red AC, Amaranth, Azorubine, Brilliant Black PN, Brilliant Blue FCF, Brown HT, Fast Green FCF, Fast Red E, Green S, Indigotine, Lithol Rubine BK, Patent Blue V, Ponceau 4R, Quinoline Yellow, Sunset Yellow FCF and Tartrazine – along with the analytical methods used, in order to update specifications.

Black carrot extract

To proceed with the assessment of black carrot extract, at least a 90-day toxicological study on a well-characterized extract representative of the material of commerce would be required.

In addition, the specifications were made tentative pending the submission of further information on the material of commerce, including a full characterization of the proteins, carbohydrates, lipids, fibre, minerals and non-anthocyanin polyphenol components in five lots each of the liquid and powder forms of black carrot extract.

Carotenoids (provitamin A)

The Committee noted that the use levels of β -carotene and β -apo-8'-carotenal provided by the sponsor were much lower than the corresponding maximum permitted levels as specified in the Codex GSEFA, and that the sponsor indicated that the majority of the maximum permitted levels are not justifiable from a technological point of view. Also, use levels were not provided for all authorized food categories. The Committee recommended that the Codex Alimentarius Commission should review current uses of β -carotene (synthetic β -carotene, β -carotene from *Blakeslea trispora* and β -carotene-rich extract from *Dunaliella salina*) and β -apo-8'-carotenal in the GSEFA, including the maximum permitted levels and the food categories in which these food additives may be used.

Gellan gum

The specifications were made tentative pending submission of new methods for characterizing the three forms of gellan gum in commerce by 2021. Specific information required is as follows:

- A method to differentiate the three commercial forms of gellan gum – i.e. high-acyl, low-acyl and low-acyl clarified.

- A method to determine the degree of acylation.
- Validation data for the above methods, including detailed description of the sample preparation.
- Data from five non-consecutive commercial batches of material using the proposed validated methods for all three forms of gellan gum.

Rosemary extract

Studies on the developmental toxicity of rosemary extract and studies to elucidate whether the effects noted on pup thyroid hormone levels can be replicated were identified as research needs to complete the evaluation. The Committee requests that this information be provided by the end of 2021.

Acknowledgements

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

FAO and WHO wish to acknowledge the significant contributions of the experts, as well as their institutions (where relevant), to the work of the eighty-seventh meeting of JECFA.





Corrigenda

The following requests for corrections, reported to the JECFA secretariats, were evaluated by the eighty-seventh JECFA meeting and found to be necessary.

- *The following corrections will be made only in the online database for specifications:*

Food additive	Original text	New text	Additional information
Copper sulfate (INS 519)	CAS: 7758-98-7	CAS: 7758-99-8	Original CAS number is for anhydrous form; however, the specifications are for the pentahydrate
Magnesium dihydrogen diphosphate (INS 450(ix))	METHOD OF ASSAY The determination of phosphorus contains the following formula $P_2O_5, \%w/w = P\% \times 4.983$	METHOD OF ASSAY The determination of phosphorus contains the following formula $P_2O_5, \%w/w = P\% \times 2.2921$	Original formula did not account for the presence of two phosphorus atoms per molecule
Basic methacrylate copolymer (INS 1205) Will also be applied to anionic methacrylate copolymer (INS 1207) and neutral methacrylate copolymer (INS 1206)	In section Definition: "Basic methacrylate copolymer is used as a coating and glazing agent for food supplements and foods for special medical purposes."	Sentence deleted.	Deletion requested by CCFAS1 ⁷ ; sentence provided only marginal information
2-Acetyl-1-pyrroline (JECFA No. 1604)	CAS: 99583-29-6	CAS: 85213-22-5	Correction to CAS number

- *The following name was missing from the List of participants in the meeting report of the eighty-sixth meeting of JECFA (WHO Technical Report Series, No. 1014, 2019):*

Dr E. Dessipri, European Directorate for the Quality of Medicines & HealthCare, Council of Europe, Strasbourg, France (*Member*)

- *The following participants were indicated as not attending the eighty-sixth meeting, but actually participated in the meeting by video conference:*

⁷ http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?Ink=1&url=https%253A%252F%252F-workspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-711-51%252FReport%252FREP19_FAE.pdf

Dr M. DiNovi, Office of Food Additive Safety, Center for Food Safety and Applied Nutrition, United States Food and Drug Administration, College Park, Maryland, USA (*WHO Temporary Adviser*)

Dr J.R. Srinivasan, Office of Food Additive Safety, Center for Food Safety and Applied Nutrition, United States Food and Drug Administration, College Park, Maryland, USA (*FAO Expert*)

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2. Report of the 51st Session of the Codex Committee on Food Additives, Jinan, China, 25–29 March 2019. Rome, Italy: Food and Agriculture Organization of the United Nations; and Geneva, Switzerland: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2019 (REP19/FA).
3. Codex General Standard for Food Additives. Rome, Italy: Food and Agriculture Organization of the United Nations; and Geneva, Switzerland: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2018 (CODEX STAN 192-1995).
4. Class names and the International Numbering System for Food Additives. Rome, Italy: Codex Alimentarius Commission, Codex Committee on Food Additives and Contaminants, Joint FAO/WHO Food Standards Programme; 2015 (CAC/GL 36-1989; http://www.fao.org/tempref/codex/Meetings/CCFAC/ccfac31/INS_e.pdf).
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7. Report of the 50th Session of the Codex Committee on Food Additives, Xiamen, China, 26–30 March 2018. Rome, Italy: Food and Agriculture Organization of the United Nations; and Geneva, Switzerland: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2018 (REP18/FA).



Annex 1

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

1. General principles governing the use of food additives (First report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 15, 1957; WHO Technical Report Series, No. 129, 1957 (out of print).
2. Procedures for the testing of intentional food additives to establish their safety for use (Second report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 17, 1958; WHO Technical Report Series, No. 144, 1958 (out of print).
3. Specifications for identity and purity of food additives (antimicrobial preservatives and antioxidants) (Third report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as Specifications for identity and purity of food additives, Vol. I. Antimicrobial preservatives and antioxidants, Rome, Food and Agriculture Organization of the United Nations, 1962 (out of print).
4. Specifications for identity and purity of food additives (food colours) (Fourth report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as Specifications for identity and purity of food additives, Vol. II. Food colours, Rome, Food and Agriculture Organization of the United Nations, 1963 (out of print).
5. Evaluation of the carcinogenic hazards of food additives (Fifth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 29, 1961; WHO Technical Report Series, No. 220, 1961 (out of print).
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230. Evaluation of certain food additives (Eighty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 1000, 2016.
231. Compendium of food additive specifications. FAO JECFA Monographs 19, 2016.
232. Safety evaluation of certain food additives. WHO Food Additives Series, No. 73, 2017.
233. Evaluation of certain contaminants in food (Eighty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 1002, 2017.
234. Evaluation of certain food additives (Eighty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 1007, 2017.
235. Safety evaluation of certain contaminants in food. WHO Food Additives Series, No. 74, FAO JECFA Monographs 19 bis, 2018.
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Annex 2

Toxicological and dietary exposure 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
Black carrot extract	N ^a , T ^b	<p>The Committee concluded that the effects observed with one anthocyanin-containing test material cannot be extrapolated to another anthocyanin-containing test material. This is because the test articles used in metabolism and toxicity studies are very heterogeneous and often not fully described and/or the anthocyanin content of the test material is too low and variable.</p> <p>Only one genotoxicity study was available for black carrot extract. Owing to the lack of toxicological data on black carrot extract, the Committee was not able to draw conclusions on its safety. To proceed with its assessment, at least a 90-day toxicological study on a well-characterized extract representative of the material of commerce would be required.</p> <p>The Committee concluded that the total mean dietary exposure to anthocyanins from naturally occurring sources and added black carrot extract ranges from 0.1 to 1.9 mg/kg body weight (bw) per day for adults (18+ years) and from 0.1 to 5.3 mg/kg bw per day for children (<18 years). The Committee noted that the contribution of the use of the food colour itself to the total mean dietary exposure to anthocyanins including from naturally occurring sources is as high as 25%.</p> <p>The Committee noted that the ADI for grape skin extract established by the previous Committee in 1982 was not reconsidered as part of this assessment and remains unchanged.</p>
Brilliant Black PN	R ^c	<p>The Committee concluded that the newly available information does not give reason to revise the previously established ADI of 0–1 mg/kg bw based on a short-term toxicity study in pigs. The Committee therefore retained the ADI for Brilliant Black PN.</p> <p>The Committee noted that the range of estimated dietary exposures for Brilliant Black PN was below the upper end of the ADI and concluded that dietary exposure to Brilliant Black PN does not present a safety concern.</p>
Carotenoids (provitamin A)	R ^d	<p>The Committee reaffirmed the conclusion from the eighty-fourth meeting that rats are not an appropriate model for deriving an ADI for β-carotene due to the relatively low bioavailability of β-carotene in rats compared with humans. Therefore, the Committee withdrew the two group ADIs of 0–5 mg/kg bw for (1) the sum of the synthetic carotenoids β-carotene, β-apo-8'-carotenal and β-apo-8'-carotenoic acid methyl and ethyl esters and (2) synthetic β-carotene and</p>

(continued)

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions
		<p>β-carotene derived from <i>Blakeslea trispora</i>, which were based on a no-observed-adverse-effect level (NOAEL) from a rat study.</p> <p>The Committee considered that no adverse health effects were observed in the general population in large, well-conducted human intervention studies in which healthy participants were administered 20–50 mg β-carotene per day for up to 12 years, in addition to background exposure from the diet.</p> <p>An additional elevated risk of lung cancer and total mortality was seen in heavy smokers (at least one pack per day) and asbestos workers in intervention studies in which participants were administered 20 mg β-carotene per day for 5–8 years or 30 mg β-carotene per day and 25 000 IU vitamin A for 5 years. The Committee noted that a generally accepted explanation for the cause of these effects has not been identified. The Committee was unable to reach any conclusion about risk from β-carotene exposure in heavy smokers.</p> <p>For the remainder of the general population, the Committee concluded that the estimated high exposure to β-carotene of 9 mg/day for a 30 kg child and 6 mg/day for a 60 kg adult from its current uses as a food additive, in addition to background exposure from the diet, would not be expected to be a safety concern. This conclusion includes synthetic β-carotene, β-carotene derived from <i>B. trispora</i> and β-carotene-rich extract from <i>Dunaliella salina</i>.</p> <p>The Committee was unable to establish a group ADI for synthetic β-carotene, β-carotene derived from <i>B. trispora</i>, β-carotene-rich extract from <i>D. salina</i>, and β-apo-8'-carotenoid acid methyl and ethyl esters because a group ADI is applicable to the general population, which includes heavy smokers. The Committee noted that it is very unlikely that it will ever be possible to establish a group ADI because further data from the population of heavy smokers cannot be gathered ethically.</p> <p>Because β-apo-8'-carotenoid acid methyl and ethyl esters were previously evaluated on the basis of β-carotene and because no new data were submitted, the Committee was unable to complete an evaluation on β-apo-8'-carotenoid acid methyl and ethyl esters.</p> <p>The present Committee established an ADI of 0–0.3 mg/kg bw for β-apo-8'-carotenol on the basis of a NOAEL of 30 mg/kg bw per day in a 13-week study in rats and application of an uncertainty factor of 100. An additional uncertainty factor to take into account the short duration of the study was not considered necessary because kidney and liver effects observed in the 13-week study at 100 mg/kg bw per day were not observed in a 2-year study at 40 mg/kg bw per day, the single dose tested.</p> <p>Estimated dietary exposure to β-apo-8'-carotenol of 0.3 mg/kg bw per day was at the upper end of the ADI established by the Committee (i.e. 0–0.3 mg/kg bw per day). The Committee noted that the estimated dietary exposure is overestimated and concluded that the current use of β-apo-8'-carotenol as a food additive will not pose a safety concern.</p>

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions
Gellan gum	R ¹ , T ²	<p>Available studies confirm the absence of any adverse effects arising from exposure to gellan gum. The Committee retained the previously established ADI “not specified”^{2b} for gellan gum.</p> <p>The Committee evaluated low-acyl clarified gellan gum for use in formulas for special medical purposes for infants. Based on a NOAEL of 100 mg/kg bw per day, the highest dose of low-acyl clarified gellan gum tested in a 21-day neonatal pig study, which modelled the 0- to 12-week period of development in human infants, and the high estimate of dietary exposure of infants to gellan gum of 13 mg/kg bw per day (based on the requested maximum concentration of gellan gum of 50 mg/L and the high level of consumption of infant formula of 260 mL/kg bw per day), a margin of exposure of 7.7 was calculated.</p> <p>The Committee concluded on the basis of several considerations (e.g. the low toxicity of gellan gum, the NOAEL being the highest dose tested, clinical studies in preterm infants and post-marketing surveillance data showing that gellan gum is well tolerated) that the margin of exposure of 7.7 calculated for the use of gellan gum in formulas for special medical purposes for infants and liquid fortification products for addition to human milk or infant formula at a maximum level of 50 mg/L in the fed product indicates low risk for the health of infants, including preterm infants, and that its proposed use is therefore of no safety concern. This conclusion applies only to the use of low-acyl clarified gellan gum. The Committee recognizes that there is variability in medical conditions among infants requiring these products and that these infants would normally be under medical supervision.</p>
Potassium polyaspartate	N	<p>In vitro data suggest that the systemic bioavailability of potassium polyaspartate is low and that potassium polyaspartate would not be cleaved in the stomach or the intestine. The NOAEL in a 90-day rat study on potassium polyaspartate was 1000 mg/kg bw per day, the highest dose tested. There was no concern for genotoxicity.</p> <p>Potassium has been evaluated by the Committee in the course of its previous evaluation of potassium hydroxide, and the result of the evaluation was an ADI “not limited”¹. Exposure to potassium that results from the use of potassium polyaspartate in wine would be within normal daily variation of background potassium exposure from the diet.</p> <p>Should microbial fermentation in the human colon occur, there would be potential exposure to L- and D-aspartic acid. L-Aspartic acid is a normal constituent of dietary protein, and systemic exposure to L-aspartic acid from the diet is much higher than potential exposure from the use of potassium polyaspartate in wine.</p> <p>There are no relevant toxicological data on D-aspartic acid. In three studies, rats exposed to around 130 mg/kg bw per day showed effects on sex hormone levels. However, NOAELs have not been identified in these studies due to the use of single doses. The Committee noted that there is a margin of exposure of more than 100-fold between the potential human dietary exposure to D-aspartic acid of up to 0.8 mg/kg bw per day and the effect level of 130 mg/kg bw per day.</p>

(continued)

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions
		<p>The estimated dietary exposure to D-aspartic acid from typical use of potassium polyaspartate in wine (up to 0.8 mg/kg bw per day) would be expected to be lower than the exposure from non-added sources in the diet. The Committee noted that it had limited data on concentrations of D-aspartic acid in food, but that food processing (e.g. heat treatment of protein, fermentation) will result in partial conversion of L-aspartic acid to D-aspartic acid.</p> <p>The Committee concluded that the use of potassium polyaspartate in wine at the maximum proposed use level of 300 mg/L is not of safety concern.</p>
Rosemary extract	R ⁱ	<p>The Committee concluded that the new studies provided evidence for the absence of reproductive toxicity, but not for the absence of developmental toxicity. The Committee retained the temporary ADI of 0–0.3 mg/kg bw, pending the submission of studies on the developmental toxicity of rosemary extract and studies to elucidate whether the effects noted on rodent pup thyroid hormone levels can be replicated. The temporary ADI will be withdrawn if the requested studies are not submitted by the end of 2021.</p> <p>Estimated mean and high-percentile dietary exposures to carnosic acid plus carnosol from use of rosemary extract as a food additive for all countries assessed based on typical use levels did not exceed the upper end of the temporary ADI (0–0.3 mg/kg bw per day). The Committee noted that when dietary exposures from naturally occurring sources are combined with dietary exposures from added sources at typical use levels, the estimated dietary exposures for children were up to 0.42 mg/kg bw per day, which exceeds the ADI. The Committee also noted that the temporary ADI is based on the highest dose tested in a short-term toxicity study in rats and that in the newly submitted reproductive/developmental toxicity screening study, no effects on reproductive toxicity or on parental animals were observed at 316 mg/kg bw per day, the highest dose tested. Therefore, the Committee does not consider the slight exceedance of the ADI to be a safety concern.</p>

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

^a For the spray-dried powder form of black carrot extract.

^b The specifications were made tentative pending further information on the material of commerce, including a full characterization of the proteins, carbohydrates, lipids, fibre, minerals and non-anthocyanin polyphenol components in five lots each of the liquid and powder forms of black carrot extract.

^c Analytical methods for determining subsidiary colouring matters and organic compounds other than colouring matters were replaced with more specific and sensitive high-performance liquid chromatography methods. The existing titrimetric method for the assay of Brilliant Black PN was replaced with a visible spectrophotometric method.

^d The specifications for synthetic β -carotene, β -carotene from *B. trispora* and β -apo-8'-carotenal were revised to replace an identification test for carotenoids with additional spectrophotometric requirements. Based on the arsenic levels from several batches of the product of commerce for β -carotene-rich extract from *D. salina*, the existing specifications for arsenic were revised from 1 mg/kg to 3 mg/kg.

^e The Committee was aware that two group ADIs for carotenoids had been established at previous meetings and that synthetic β -carotene had been included in both group ADIs. The Committee speculated that the Committee at the fifty-seventh meeting did not recognize that synthetic β -carotene was already part of a group ADI and included it in a new group ADI.

^f The Committee concluded that the use of ethanol in the manufacturing of gellan gum is not a safety concern when used according to good manufacturing practice. The specification for ethanol was removed.

^g The specifications were made tentative, pending submission of new methods for characterizing the three forms of gellan gum in commerce by 2021.

^h 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.

^h Now called an ADI "not specified" (see table note h).

^j The Committee removed the specification for ethanol, and the tentative status of the specifications for rosemary extract was removed.

Food additives considered for specifications only

Food additive	Specifications
Cassia gum	T ^a
Citric and fatty acid esters of glycerol (CITREM)	R ^b
Metatartaric acid	R ^c
Mannoproteins from yeast cell walls	R ^d
Steviol glycosides	See note e

R: existing specifications revised; T: tentative specifications

^a At the eighty-sixth meeting, the Committee updated the specifications for cassia gum by including the high-performance liquid chromatography method received and removed their tentative status. Based on comments received about the method performance, the present Committee reviewed the method again and noted that additional investigations were required. Therefore, the Committee decided to make the specifications tentative until ongoing investigations are completed.

^b The Committee received a suitable validated replacement method for an obsolete packed column gas chromatographic method for the determination of total citric acid content, along with performance characteristics of the method and data on the total citric acid content in products currently available in commerce, determined using that method. The Committee included the new method in the specifications and deleted the previous method. A new high-performance liquid chromatography method for the analysis of glycerol, supported by validation data, was provided and included in the revised specifications. The limit for glycerol was maintained. Data on the use of additional neutralizing salts in CITREM manufacture were received and added to the specifications. The lead limit for use of CITREM in infant formula was corrected to 0.5 mg/kg according to the previous evaluation. Data on the sulfated ash levels and the content of minerals in neutralized CITREM products were provided. The limit for sulfated ash was maintained for non-neutralized CITREM, and new limits were set for partially neutralized and for wholly neutralized CITREM. The tentative status of the specifications was removed.

^c The Committee received information on optical rotation, infrared identification, free tartaric acid content, degree of esterification and molecular weight distribution, together with the analytical methods. The Committee revised the specifications for free tartaric acid, optical rotation, molecular weight and molecular weight distribution and included a specification for polydispersity index. The tentative status of the specifications for metatartaric acid was removed.

^d The Committee revised the specifications monograph and noted that a change in the name of the food additive from "Yeast extracts containing mannoproteins" to "Mannoproteins from yeast cell walls" was appropriate. The Committee noted that all mannoproteins, regardless of the range of molecular weights, were included in the same specifications monograph and therefore specifying a range of average molecular weight and a method for measuring it was not essential. Data were also received for metallic impurities. The Committee reviewed the information received and decided that only a limit for lead was required. The tentative status of the specifications was removed.

^e A framework was adopted for developing specifications for steviol glycosides by four different methods of production. Specifications for steviol glycosides produced by different production methods were included as annexes, as below:

- Annex 1: Steviol Glycosides from *Stevia rebaudiana* Bertoni (**revised** from the specifications monograph for Steviol glycosides from *Stevia rebaudiana* Bertoni prepared at the eighty-fourth meeting of JECFA (INS 960a)).
- Annex 2: Steviol Glycosides from Fermentation (specifications for Rebaudioside A from multiple gene donors expressed in *Yarrowia lipolytica* (INS 960b(ii)) prepared at the eighty-second meeting of JECFA were **revised** to include other steviol glycosides from *Saccharomyces cerevisiae* and *Yarrowia lipolytica*).
- Annex 3: Enzyme Modified Steviol Glycosides (**new** specifications).
- Annex 4: Enzyme Modified Glucosylated Steviol Glycosides (**new** specifications, **tentative** pending further information concerning the analytical methods).

Flavouring agents considered for specifications only

Flavouring agent	No.	Specifications
Methyl propionate	141	R ^a
Ethyl oleate	345	R ^b
alpha-Methyl-beta-hydroxypropyl alpha-methyl-beta-mercaptopropyl sulfide	547	R ^c
Vanillin	889	R ^d
Ethyl vanillin	893	R ^e
2,2,3-Trimethylcyclopent-3-en-1-yl acetaldehyde	967	R ^f
alpha- and beta-Cyclocitral (50:50 mixture)	979	R ^g

(continued)

Flavouring agent	No.	Specifications
Sodium 2-(4-methoxyphenoxy)propanoate	1029	R ^h
2,2,6-Trimethyl-6-vinyltetrahydropyran	1236	R ⁱ

R: existing specifications revised

^a The Committee revised the specific gravity to 0.912–0.918.

^b The Committee revised the assay minimum to not less than 75% ethyl oleate. Specifications for the secondary components were also established: ethyl linoleate (3.4–11.5%), ethyl palmitate (0.4–5.1%), ethyl stearate (0.5–2.5%), ethyl laurate (1–2%) and other fatty acid ethyl esters.

^c The Committee revised the refractive index to 1.512–1.522, the specific gravity to 1.040–1.050 and the assay minimum to 95%.

^d The Committee revised the melting point to 81–84 °C.

^e The Committee revised the melting point to 76–79 °C.

^f The Committee revised the assay minimum to 93%, with a secondary component of up to 2% of gamma-campholenic aldehyde.

^g The Committee revised the specifications to include the Chemical Abstracts Service (CAS) numbers for alpha-cyclocitral (CAS No. 432-24-6) and for the mixture of alpha- and beta-cyclocitral (CAS No. 52844-21-0). The Flavis and Council of Europe (COE) numbers for alpha- and beta-cyclocitral were also included. The refractive index range was revised to 1.4986–1.4991.

^h The Committee revised the CAS number (150436-68-3) and Flavis number (08.127) to reflect the salt form. The melting point was revised to 184–190 °C. Identifiers and synonyms associated with the free acid were removed.

ⁱ The Committee changed the minimum assay to 95%, the refractive index to 1.442–1.452 and the specific gravity to 0.863–0.873.

Annex 3

Secondary components for flavouring agents with revised specifications with minimum assay values of less than 95%

JECFA No.	Flavouring agent	Minimum assay value	Secondary components	Comments on secondary components
345	Ethyl oleate	75%	Ethyl linoleate (3.4–11%), ethyl palmitate (0.4–5.1%), ethyl stearate (0.5–2.5%), ethyl laurate (1–2%) and other fatty acid ethyl esters	The impurities are fatty acids with similar structures. As such, there are no safety concerns at current levels when occurring as secondary components in JECFA No. 345 when used as a flavouring agent.
967	2,2,3-Trimethylcyclopent-3-en-1-yl acetaldehyde	93%	Gamma-campholenic aldehyde (2,2,4-trimethyl-cyclopent-3-en-1-yl acetaldehyde) (2%)	The impurity is a positional isomer of JECFA No. 967, with similar toxicity. As such, there are no safety concerns at current levels when occurring as a secondary component in JECFA No. 967 when used as a flavouring agent.



Annex 4

Meeting agenda



Food and Agriculture
Organization of the
United Nations



World Health
Organization

87th JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES (JECFA)
FAO Headquarters, Rome, 4 – 13 June 2019

Opening:

Philippine Room (C277) 4 June at 9.30h

Draft Agenda

1. Opening
2. Declarations of Interests (information by the Secretariat on any declared interests and discussion, update by experts)
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:

- Black carrot extract
- Brilliant Black PN (INS 151)
- Carotenoids (INS 160x):
 - * β -carotene
 - * β -carotene from *Blakeslea trispora*

- * β -apo-8'-carotenal
 - * β -apo-8'-carotenoic acid methyl and ethyl esters
 - Gellan gum (INS 418)
 - Potassium polyaspartate
 - Rosemary extract (INS 392)
- 7.2. Steviol glycosides - Establishment of a framework for safety assessment of steviol glycosides produced by different technologies
- 7.3. Food additives for revision of specifications and analytical methods:
- β -Carotene-rich extract from *Dunaliella salina*
 - Metatartaric acid (INS 353)
 - Yeast extracts containing mannoproteins
 - Steviol Glycosides (Rebaudioside M manufactured from two strains of yeast from the *Saccharomyces* family)
 - Steviol Glycosides (Rebaudioside A and M, respectively, from Multiple Gene Donors Expressed in *Yarrowia lipolytica*) (INS 960)
 - Steviol glycosides (Steviol Glycosides, Rebaudioside A, Rebaudioside D, Rebaudioside M; Enzyme Modified Steviol Glycosides, Enzyme Modified Stevia Leaf Extract)
- 7.4. Establishment of specifications for certain flavouring agents
- Vanillin (JECFA No. 889)
 - Ethyl vanillin (JECFA No. 893)
 - Methyl propionate (JECFA No. 141)
 - 2,6,6-Trimethyl-1&2-cyclohexen-1-carboxaldehyde (JECFA No. 979)
 - Sodium 2-(4-methoxyphenoxy)propanoate (JECFA No. 1029)
 - 2,2,3-Trimethylcyclopent-3-en-1-yl acetaldehyde (JECFA No. 967)
 - Ethyl oleate (JECFA No. 345)
 - 2,2,6-Trimethyl-6-vinyltetrahydropyran (JECFA No. 1236)
 - alpha-Methyl-beta-hydroxypropyl alpha-methyl-beta-mercaptopropyl sulfide (JECFA No. 547)
8. Other matters to be considered (general considerations)
- Update of EHC240:
- For discussion
- Refinement of criteria for establishing group ADI and ADI not specified
 - Proposal for updated guidance on evaluation of enzyme preparations
- For consideration
- Proposal for updated guidance on evaluation of genotoxicity studies
 - Update of Chapter 5 in EHC240 on dose-response modelling and application of the benchmark-dose approach
9. Errata

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 food additives

Eighty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 1014, 2019 (156 pages)

Evaluation of certain veterinary drug residues in food

Eighty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 1008, 2017 (150 pages)

Safety evaluation of certain food additives

Eighty-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives
WHO Food Additives Series, No. 75, 2018 (244 pages)

Evaluation of certain food additives

Eighty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 1007, 2017 (92 pages)

Safety evaluation of certain contaminants in food

Eighty-third meeting of the Joint FAO/WHO Expert Committee on Food Additives
WHO Food Additives Series, No. 74, 2018 (897 pages)

Evaluation of certain contaminants in food

Eighty-third report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 1002, 2017 (166 pages)

Safety evaluation of certain food additives

Eighty-second meeting of the Joint FAO/WHO Expert Committee on Food Additives
WHO Food Additives Series, No. 73, 2017 (493 pages)

Evaluation of certain food additives

Eighty-second report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 1000, 2016 (162 pages)

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)

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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 and to prepare specifications for the identity and purity of the food additives, including flavouring agents.

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. A summary follows of the Committee's evaluations of technical, toxicological and dietary exposure data for six food additives or groups of food additives: black carrot extract; Brilliant Black PN; carotenoids (provitamin A); gellan gum; potassium polyaspartate; and rosemary extract.

Specifications for the following food additives were revised: citric and fatty acid esters of glycerol (CITREM); metatartaric acid; mannoproteins from yeast cell walls; and steviol glycosides. Specifications for cassia gum were made tentative.

Specifications for eight flavouring agents were revised: methyl propionate; ethyl oleate; alpha-methyl-beta-hydroxypropyl alpha-methyl-beta-mercaptopropyl sulfide; vanillin; ethyl vanillin; 2,2,3-trimethylcyclopent-3-en-1-yl acetaldehyde; alpha- and beta-cyclocitral (50:50 mixture); sodium 2-(4-methoxyphenoxy)propanoate; and 2,2,6-trimethyl-6-vinyltetrahydropyran.

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

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