WHO Technical Report Series

1027

Evaluation of certain food additives

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

Food and Agriculture Organization of the United Nations

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List of abbreviations and acronyms

ADME	absorption, distribution, metabolism and excretion
ADI	acceptable daily intake
AMP	adenosine 5´-monophosphate
ATCC	American Type Culture Collection
BLAST	basic local alignment search tool
bw	body weight
CAS	Chemical Abstracts Service
CCFA	Codex Committee on Food Additives
CHD-FA	carbohydrate-derived fulvic acid
CIFOCOss	Chronic Individual Food Consumption database summary statistics
COVID-19	coronavirus 2019
EFSA	European Food Safety Authority
EHC	Environmental Health Criteria
FAO	Food and Agriculture Organization of the United Nations
GEMS/Food	Global Environment Monitoring System, Food Contamination Monitoring and Assessment Programme
GIFT	Global Individual Food consumption data Tool
GSFA	General Standard for Food Additives
ICP-AES	inductively coupled plasma–atomic emission spectrometry
INS	International Numbering System
IPRU	inositol phosphate releasing unit
IPTG	isopropyl β-D-thiogalactopyranoside
JECFA	Joint Expert Committee on Food Additives
MOE	margin of exposure
MPL	maximum permitted level
MSDI	maximized survey-derived intake
NOAEL	no-observed-adverse-effect-level
OECD TG	Organisation for Economic Co-operation and Development test guideline
PI-PLC	phosphatidylinositol-specific phospholipase C
PVOH	polyvinyl alcohol
SEF	sucrose ester of fatty acids
SOE	sucrose oligoester
SPET	single portion exposure technique
TMDI	theoretical maximum daily intake
TOS	total organic solids
TRS	Technical Report Series
U	unit
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. 80, 2020.

Specifications are issued separately by FAO under the title: *Compendium of food additive specifications*. FAO JECFA Monographs 25, 2020.

1. Introduction

The FAO/WHO Joint Expert Committee on Food Additives (JECFA) met by video-conference on 1–12 June 2020. The meeting was opened on behalf of the Director-General of the World Health Organization (WHO) by Dr Francesco Branca, Director, Department of Nutrition and Food Safety (WHO), and on behalf of the Director-General of the Food and Agriculture Organization of the United Nations (FAO) by Dr Markus Lipp, Head of Food Safety and Quality, Agriculture and Consumer Protection Department, FAO. Dr Branca welcomed all meeting participants and described the roles and responsibilities of JECFA in the international work of the Codex Alimentarius Commission in developing food safety standards. He reminded the JECFA experts of their responsibility to elaborate the least biased, best scientific advice possible. Dr Lipp reminded participants that they had been invited not as representatives of their employers or countries but in their capacity as scientific experts for providing sound, independent scientific advice as a basis for food standards designed to protect the health of all consumers and trade among all regions and countries. He urged the attendees to be as open and transparent as possible, emphasizing that scientific excellence requires input from all and the courage to ask critical questions.

1.1 Procedural matters

The 89th meeting of JECFA was originally scheduled for 2–11 June 2020 at WHO headquarters in Geneva, Switzerland. Because of the travel restrictions and lock-downs due to the COVID-19 pandemic in many countries, however, the joint FAO/WHO JECFA secretariat was unable to convene the meeting as scheduled. The secretariat evaluated possible alternatives, including cancelling the meeting, but, to avoid a delay in delivering the requested scientific advice to the Codex Alimentarius Commission, the secretariat decided to hold the meeting online by video-conferencing. In view of the countries of origin of the invited experts, the only possible time slot for a video-conference was restricted to 12:00–16:00 CEST each day. This allowed approximately 40% of the usual daily time (8–10 h) of a JECFA 8-day face-to-face meeting.

The FAO/WHO JECFA secretariat contacted all the invited experts and the Codex secretariat to discuss changes to the meeting format. The experts expressed their willingness and availability to participate remotely in the meeting as a one-time measure because of the exceptional circumstances of the COVID-19 pandemic. They agreed to extend the duration of the meeting by 2 days, adding Monday 1 June and Friday 12 June 2020; however, their commitments did not allow extension of the meeting into the week before or after those scheduled.

After discussion, the experts and the FAO/WHO JECFA secretariat decided that the 89th JECFA could evaluate only the compounds that are listed in the final table of contents because of the shortage of time. Nisin (INS 234), natamycin (INS 235), β-glucanase from *Streptomyces violaceoruber* expressed in *S. violaceoruber*, collagenase from *S. violaceoruber* expressed in *S. violaceoruber*, phosphodiesterase from *Penicillium citrinum* and phospholipase A2 from *S. violaceoruber* expressed in *S. violaceoruber*, which had been scheduled for discussion, were therefore not considered. During the meeting, it became apparent that two further evaluations could not be completed – that for alicyclic ketones, secondary alcohols and related esters and a toxicological evaluation of riboflavin from *Ashbya gossypii*. All these compounds will be re-scheduled for evaluation at future JECFA meetings.

The 89th JECFA meeting was held on an online platform on 1–12 June 2020. While the experts participated fully, they noted that an online meeting does not facilitate the usual interaction among experts, within and across the WHO and FAO sub-groups. The experts considered that the success of the 89th meeting was due to a large extent to the cohesion among the experts that resulted from the trust generated during previous face-to-face meetings.

The experts decried the significant difficulty of meeting informally outside the scheduled meeting times because of the large differences in time zones. They noted that such informal interactions during physical meetings are instrumental to solving problems and to discussing issues in depth, bilaterally or in small groups, and added that informal meetings often gave rise to solutions to challenging problems. The inability to have such meetings was considered to have hindered progress at the current meeting and led to less efficient use of experts' time.

The experts emphasized further that an invitation to a physical JECFA meeting at FAO or WHO headquarters gives rise to significant recognition by the expert's employer of the weight and reach of the outcomes and the responsibility and workload required for full participation in a JECFA meeting. The lack of recognition of the workload and of the significance of participation in a JECFA meeting led to an increase in other demands on experts, resulting in notable distraction, with more frequent scheduling conflicts. The experts concluded that, cumulatively, such factors would be significantly counterproductive for participation in future virtual JECFA meetings and for the efficiency of such meetings.

While the collaborative software solutions provided by FAO and WHO made the meeting possible, the experts urged FAO and WHO to explore means to improve the stability of the platforms used; significant meeting time was lost due to slow responses of both systems. Furthermore, the stability, reliability and

consistency of the experts' Internet services did not at times fulfil the minimum requirements necessary for effective participation in the meeting, as frequent disconnections and slow transmission of shared content were an issue for some experts.

In recognition of the difficulties and the tremendous effort made, the joint FAO/WHO secretariat expresses its deep gratitude to all the experts for their commitment and flexibility, not least as the scheduled meeting times were inconvenient for many.

1.2 Declarations of interests

The Joint Secretariat informed the Committee that all the experts participating in the 89th JECFA meeting had completed declaration of interest forms, and no conflicts of interest were identified.

1.3 Adoption of the agenda

The draft agenda was adopted.

2. General considerations

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. 80, 2020. Specifications are issued separately by FAO under the title: Compendium of food additive specifications. FAO JECFA Monographs 20, 2020.

2.1 Update on revised guidance documents for Environmental Health Criteria 240

2.1.1 **Update of guidance on dose–response assessment and derivation of health-based guidance values** (revision of Environmental Health Criteria (EHC) 240, Chapter 5)

Since the last update to the Committee in June 2019 (WHO Technical Report Series (TRS) No. 1020, 2019), revision of Chapter 5 of EHC 240, on dose–response assessment and derivation of health-based guidance values, has continued, and a draft of the chapter was sent for public consultation in December 2019. In response, the Secretariat received about 300 comments from 12 organizations or individuals, indicating a high level of interest. The comments included many helpful suggestions for further revision and clarification of the text. Most of the comments have now been considered and addressed, and the work will be completed soon. After editing, the text will be published online as an updated chapter of EHC 240.

2.1.2 **Update of guidance on evaluation of enzyme preparations** (revision of EHC 240, Chapter 9.1.4.2)

The Committee was given an update on progress made in revising guidance on the evaluation of enzymes for use in food. An expert working group was established in 2018 to discuss the available information on the safety of enzymes used in food and current practices of the food enzyme industry. Several documents and definitions were amended and submitted for public comment late in 2019. The comments received were evaluated, and the text of a revised version of Chapter 9.1.4.2 of EHC 240 was edited further as necessary. It is reproduced in Annex 1 to this document.

The working group made a series of recommendations to this Committee, which came to the following consensus.

1a. The Committee adopted the proposed definitions of "safe food enzyme production strain" and "presumed safe progeny strain" (section 2) with minor editorial changes.

1b. The Committee adopted the proposed revisions to Chapter 9.1.4.2 of EHC 240 pertaining to enzymes, including a revision of the classification of enzymes and their definitions. The text for Class I Type iii and Class II enzymes was modified to state that "an ADI may be established."

1c. The Committee approved the proposed checklist of data requirements for the risk assessment of enzyme preparations in submissions for review by JECFA, with a change to one of the test requirements. The Committee debated the value of including on the checklist a request for information on "Bioinformatics analysis of the amino acid sequence for potential matches with known toxins" (checklist item #29). The Committee decided that it should remain on the checklist, and the usefulness of such information should be evaluated once sufficient experience has been gained.

1d. The Committee adopted the proposed list of terms and definitions related to submissions on enzyme preparations for use in food and added a definition of "total organic solids".

2) The Committee recommended that allergenicity should be assessed only for enzyme preparations proposed for inclusion in Class I Type iii or Class II.

3) The Committee debated whether it would be appropriate to combine consideration of immobilized enzyme preparations that are in contact with foods only during processing with consideration of enzyme preparations added to foods but removed from the final products. Differing points of view were expressed, and the Committee was reminded that such consideration did not apply to other situations in which food-grade carriers and formulation ingredients are used. Furthermore, the Committee considered that the levels of residues of immobilizing agents in the final product would be extremely low; the levels of these substances or their contaminants permitted in the final product should be at the lowest levels that are technologically feasible. The Committee decided that the wider issue of food contact materials was not one of their current terms of reference, and their consideration would have to be initiated by the Codex Alimentarius Commission or others before it could be taken up.

4) The Committee supported establishment of a separate online database for toxicological data and specifications for enzyme preparations for use in food evaluated by JECFA in order to simplify presentation of the data to users (similar to that currently used for flavourings).

5) The Committee supported establishment of a separate JECFA numbering system for identifying enzyme preparations for which JECFA had completed safety evaluations (similar to that used for flavourings).
6) The Committee supported development of an enzyme-specific template for the submission of information on analytical methods, including method performance characteristics (method validation data) and quality control data.

2.1.3 Update of guidance on evaluation of the genotoxicity of chemical substances in food

Since the last update provided to the Committee, in June 2019 (TRS 1020), on revision of Chapter 4.5 of EHC 240, guidance on evaluating the genotoxicity of chemical substances in food, a draft of the chapter was sent for public consultation in December 2019. In response, the Secretariat received about 300 comments from 14 organizations or individuals, indicating a high level of interest. The comments included many helpful suggestions for further revision and clarification of the text. Most of the comments have now been considered and addressed, and the work will be completed soon. After editing, the text will be published online as an updated chapter of EHC 240.

2.2 Other matters of interest to the Committee

Withdrawal of the ADI for lipase from Aspergillus oryzae, var.

In evaluating lipase from *Mucor javanicus* at the present meeting (item 3.1.5), the Committee noted that the specifications for lipase from *Aspergillus oryzae*, var. had been withdrawn by the Committee at its 55th meeting (Annex 2, reference *149*) but that it had not addressed the consequences of the withdrawal of specifications on its acceptable daily intake (ADI). The Committee at its current meeting decided to withdraw the ADI "not specified" for lipase from *Aspergillus oryzae*, var.

The Committee also noted that specifications for other food additives had been withdrawn at the 55th meeting without addressing the consequences for the respective ADIs. The Committee recommends reconsideration of the ADIs concerned at a future meeting.

3. Specific food additives (other than flavouring agents)

The Committee evaluated five food additives for the first time and re-evaluated one other. Four food additives were considered for revision of specifications only and one food additive for exposure only. Information on the safety evaluations and specifications is summarized in Annex 3. Details of further toxicological studies and other information required for certain substances are summarized in section 5.

3.1 Toxicological evaluation, exposure assessment and establishment of specifications

3.1.1 Adenosine 5′-monophosphate deaminase from *Streptomyces murinus*

Explanation

At the request of the Codex Committee on Food Additives (CCFA) at its Forty-ninth Session *(1)*, the Committee evaluated the safety of adenosine 5′-monophosphate deaminase (AMP deaminase; Enzyme Commission No. 3.5.4.6) from *Streptomyces murinus*, which it had not previously considered. The enzyme enhances flavour in foods by promoting the conversion of AMP into inosine 5′-monophosphate. The AMP deaminase enzyme preparation is intended for use in processing yeast and yeast-like products and in the production of flavourings.

In this report, the expression "AMP deaminase" refers to the enzyme and its amino acid sequence; the expression "AMP deaminase concentrate" refers to the enzyme concentrate used in the toxicity studies; and the expression "AMP deaminase preparation" refers to the enzyme preparation formulated for commercial use.

At its present meeting, the Committee considered the submitted data and also conducted a literature search in Google Scholar with the linked search terms "adenosine 5' monophosphate deaminase" and "Streptomyces murinus". Although 47 references were identified, none was considered relevant for this toxicological evaluation.

Genetic background

S. murinus is a non-pathogenic, non-toxigenic actinobacterium found in soil, which has been used as a source organism in the production of food enzymes. The *S. murinus* production strain AE-DNTS was obtained by chemical mutagenesis followed by selection of individual colonies of the parent *S. murinus* strain (NBRC 14802). The phylogenetic relationship of the production strain was verified by *gyrB* gene sequence analysis and BLAST homology searches.

Chemical and technical considerations

AMP deaminase is manufactured by controlled aerobic batch fermentation of a pure culture of *S. murinus* AE-DNTS carrying the AMP deaminase gene. The enzyme is secreted into the fermentation medium, separated in a series of filtration steps and concentrated. The concentrated liquid enzyme is formulated to an enzyme preparation by the addition of dextrin. The AMP deaminase enzyme preparation is produced in accordance with good manufacturing practice with food-grade raw materials. The AMP deaminase preparation is free of the production organism and any antibiotic activity and conforms to the General Specifications for Enzyme Preparations used in Food Processing (Annex 2, reference *185*).

AMP deaminase catalyses the hydrolysis of AMP to inosine monophosphate, with the release of ammonia. The AMP deaminase preparation is intended for use at levels ranging from 1 to 100 mg total organic solids (TOS) per kilogram raw material in yeast processing for cereals and in the production of flavourings of vegetable, animal or microbiological origin. Enzyme activity is determined spectrophotometrically by treating AMP substrate with AMP deaminase and measuring the difference in absorbance at 265 nm after incubation for 15 min. The activity is expressed in deaminase activity units (U); 1000 units is defined by the amount of enzyme required to decrease the absorbance by 0.1 when measured at 265 nm for 60 min. The mean activity of AMP deaminase from the three batches of deaminase concentrate provided was 144 000 000 U/g. The mean percentage TOS from the three batches of the AMP deaminase concentrate provided was 7.0%. TOS includes the enzyme of interest and residues of organic materials, such as proteins, peptides and carbohydrates, derived from the production organism during the manufacturing process. The activity of the AMP deaminase preparation in commerce is typically 60 000 000 U/g, or ~ 3.5% TOS. AMP deaminase is expected to be inactivated during processing.

Biological data

Biotransformation

No information was available.

Assessment of potential allergenicity

AMP deaminase was assessed for allergenicity by bioinformatics, consistent with the criteria recommended by FAO/WHO and others *(2,3*; Annex 2, reference *223*). A search for matches with > 35% identity in a sliding window of 80 amino acids and a search for sequence identity of eight contiguous amino acids were conducted in publicly available databases of known allergens. No matches were found. AMP deaminase is not expected to pose a risk of allergenicity.

Toxicological studies

In a 13-week oral toxicity study in rats, AMP deaminase enzyme concentrate (TOS content, 13.8%) was mixed in water and administered by gavage at doses ≤ 2000 mg/kg bw per day, equal to 275 mg TOS/kg bw per day *(4)*. Treatment-related, dose-dependent histopathological findings were observed in the lungs of both males and females and in the trachea of males at ≥ 1000 mg/kg bw per day. The Committee considered the possibility that the histopathological observations were due to gavage-related reflux *(5)* but concluded that there was insufficient information to support this interpretation of the findings. The Committee identified a no-observed-adverse-effect level (NOAEL) of 500 mg/kg bw per day, equal to 69 mg TOS/kg bw per day.

The enzyme concentrate was not genotoxic in a bacterial reverse mutation assay *(6)* or in a chromosomal aberration assay *(7)*.

Assessment of dietary exposure

The Committee evaluated one estimate of the theoretical maximum daily intake (TMDI) of the AMP deaminase enzyme preparation made with the budget method, which was provided by the sponsor. The TMDI was based on the level of TOS in the deaminase enzyme preparation and its maximum proposed use levels (≤ 100 mg TOS/kg ingredient, equivalent to ≤ 2 mg TOS/kg in the final food) and the assumption that 25% of the food supply contains the enzyme preparation. The resulting TMDI was a total of 0.075 mg TOS/kg bw per day from solid food and non-milk beverages. The Committee noted that the enzyme is inactivated in the processing of food ingredients and has no function in the final food.

Evaluation

Negative results were observed in genotoxicity tests, and a NOAEL of 500 mg/kg bw per day (equal to 69 mg TOS/kg bw per day) was identified in a 13-week oral toxicity study. Comparison of the dietary exposure estimate of 0.075 mg TOS/kg bw per day with the NOAEL of 69 mg TOS/kg bw per day gives a margin of exposure (MOE) of 920. On this basis, the Committee concluded that the AMP deaminase enzyme preparation from *S. murinus* would not pose a health concern when used in the applications specified, at the levels specified and in accordance with good manufacturing practice.

A toxicological monograph and a dietary exposure assessment were prepared.

New specifications and a chemical and technical assessment were prepared.

References

1. Report of the Forty-ninth Session of the Codex Committee on Food Additives, Macao SAR, China, 20–24 March 2017. Rome: Food and Agriculture Organization of the United Nations; (http://www.fao.org/fao-who-codexalimentarius/sh-proxy/it/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-711-49%252FReport%252FREP17_FAe.pdf, accessed 28 February 2020).

2. Evaluation of allergenicity of genetically modified foods. Report of a Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology 22–25 January 2001. Rome: Food and Agriculture Organization of the United Nations; Geneva: World Health Organization; 2001 (http://www.who.int/foodsafety/publications/gmo-allergenicity/en, accessed 28 February 2020).

3. Codex Alimentarius. Foods derived from modern biotechnology, second edition. Rome: Food and Agriculture Organization of the United Nations; Geneva: World Health Organization; 2009 (http://www.fao.org/3/a-a1554e.pdf).

4. Katsumata T. A 13-week oral study of deaminase (*Streptomycs murinus*) in rats. Study No. B6527. Final report [English translation]. Unpublished study conducted at Bozo Research Center Inc., Gotemba-shi, Shinozuoka, Japan, 2009. Submitted to WHO by Amano Enzyme Inc., Naka-ku, Nagoya, Japan.

5. Damsch S, Eichenbaum G, Tonelli A, Lammens L, Van den Bulck K, Feyen B, et al. Gavage-related reflux in rats: identification, pathogenesis, and toxicological implications (review). Toxicol Pathol. 2011;39:348–60.

6. Oguma Y. Bacterial reverse mutation test of deaminase (*Streptomyces murinus*) (Study No. T-0266). Final report [English translation]. Unpublished study conducted at Bozo Research Center Inc., Tokyo, Japan; 2008. Submitted to WHO by Amano Enzyme Inc., Naka-ku, Nagoya, Japan.

7. Sono A. Chromosome aberration test in cultured Chinese hamster cells treated with deaminase (*Streptomyces murinus*) in rats (Study No. M-1332). Final report [English translation]. Unpublished study conducted at Bozo Research Center Inc. Tokyo, Japan, 2009. Submitted to WHO by Amano Enzyme Inc., Naka-ku, Nagoya, Japan.

3.1.2 D-Allulose 3-epimerase from *Arthrobacter globiformis* M30 expressed in *Escherichia coli* K-12 W3110

Explanation

At the request of the CCFA at its Forty-eighth Session *(1)*, the Committee evaluated the safety of D-allulose 3-epimerase (Enzyme Commission No. 5.1.3.30; Chemical Abstract Services [CAS] No. 1618683-38-7) from *Arthrobacter globiformis* M30 expressed in *Escherichia coli* K-12 W3110. The Committee has not previously evaluated this enzyme preparation. The enzyme epimerizes D-fructose at the C3 position to form D-allulose, a low-calorie sweetener. The intended use of the enzyme preparation is as a processing aid in the production of D-allulose.

In this report, the expression "D-allulose 3-epimerase" refers to the enzyme itself and its amino acid sequence; the expression "D-allulose 3-epimerase concentrate" refers to the test material used in the toxicity studies evaluated; and

the expression "D-allulose 3-epimerase preparation" refers to the preparation formulated for commercial use.

The Committee considered the submitted data and also conducted a literature search in Google Scholar with the linked search terms "D-allulose 3-epimerase" and "Arthrobacter globiformis", which generated 49 references. No relevant published studies on toxicological aspects were found.

The Committee noted that this evaluation is of the safety of D-allulose 3-epimerase and not of the safety of the food additive D-allulose (also referred to as D-psicose). The Committee has never evaluated the safety of D-allulose.

Genetic background

D-Allulose 3-epimerase is produced by a genetically modified strain of *E. coli* K-12, a non-pathogenic, non-toxigenic microorganism with a history of safe use in food. The *E. coli* K-12 W3110 production strain pWKLP was prepared by transforming the *E. coli* recipient strain with an expression plasmid carrying the structural D-allulose 3-epimerase gene from an *A. globiformis* M30 donor, a D-allulose 3-epimerase gene transcription promoter, a repressor and its regulatory region important for the function of the promoter, a terminator and an antibiotic selection marker. Transformation was performed by the calcium chloride method, followed by selection of a strain for optimal production. The production strain was tested for the absence of antibiotic resistance and of any transformable rDNA. Transformation was confirmed by DNA sequencing, as was the stability of the expression plasmid.

Chemical and technical considerations

D-Allulose epimerase is manufactured by controlled aerobic batch fermentation of a pure culture of the genetically modified strain of *E. coli*. Once the main fermentation is stopped by bacteriolysis (heat and lysozyme for 18 h), the enzyme is extracted from the cell material. This is followed by a series of filtration steps; the liquid enzyme is then concentrated and purified. It is formulated into the commercial D-allulose 3-epimerase preparation by the addition of water and D-sorbitol; the powdered product is freeze-dried. The D-allulose 3-epimerase enzyme preparation conforms to the General Specifications and Considerations for Enzyme Preparations Used in Food Processing (Annex 2, reference *185*).

D-Allulose 3-epimerase activity is determined by measuring the production of fructose resulting from epimerization of an allulose substrate. One unit of D-allulose 3-epimerase activity is defined as the quantity of enzyme required to produce 1 µmol D-fructose per minute under the specified conditions. The percentage TOS in a batch of the D-allulose 3-epimerase concentrate provided was 91.5%. TOS includes the enzyme of interest and residues of organic materials

such as proteins, peptides and carbohydrates derived from the production organism during the manufacturing process. The mean activity of three batches of commercial D-allulose 3-epimerase preparation was 405 U/g, and the mean TOS was 4.7%. D-Allulose 3-epimerase is expected to be inactivated during processing and absent from D-allulose product.

Biotransformation

D-Allulose 3-epimerase was readily hydrolysed by proteolytic enzymes (pepsin and pancreatin) into small peptides in *in vitro* assays with simulated gastric fluid and simulated intestinal fluid. It is expected that ingested D-allulose 3-epimerase would be digested like other dietary proteins.

Assessment of potential allergenicity

D-Allulose 3-epimerase was assessed for potential immunological cross-reactivity with known allergens by bioinformatics, consistent with the criteria recommended by FAO/WHO *(2,3)*. A search for matches with > 35% identity in a sliding window of 80 amino acids and a search for sequence identity of eight contiguous amino acids produced no matches. Additionally, the enzyme is anticipated to be denatured and removed during purification of D-allulose; ingested enzyme is expected to be hydrolysed by digestive enzymes. On the basis of the intended use and the available information, the Committee concluded that dietary exposure to D-allulose 3-epimerase is not anticipated to pose an allergenic risk.

Toxicological studies

In a short-term (13-week) oral toxicity study in rats, no treatment-related adverse effects were observed when the dried powdered D-allulose 3-epimerase concentrate was mixed into their feed at a concentration of < 2.0%, the highest tested. This concentration corresponded to a dose of 1100 and 1300 mg TOS/kg bw per day in males and females, respectively. The Committee identified a NOAEL of 1100 mg TOS/kg bw per day, the highest dose tested. The results of a bacterial reverse mutation test and an *in vitro* micronucleus test with cultured human lymphocytes with a powder form of D-allulose 3-epimerase concentrate as the test material were both negative. The Committee concluded that the D-allulose 3-epimerase enzyme preparation is unlikely to be genotoxic.

Assessment of dietary exposure

Although estimates of dietary exposure to D-allulose were submitted, the Committee undertook its own assessment. Dietary exposure to TOS in the enzyme preparation was calculated by using D-allulose exposures from food to

determine the amount of the enzyme preparation used to produce that amount of D-allulose, then applying the proportion of TOS in the preparation. The estimated dietary exposures reported by the sponsor were considered by the Committee to be overestimates because of the method used to estimate the starting dietary exposures. The Committee chose to use dietary exposures estimated for the evaluation as mg TOS/kg bw per day, which were ≤ 0.13 for infants, children and adults at the mean, and ≤ 0.38 for infants and children and ≤ 0.31 for adults at the 90th percentile.

For the dietary exposure assessment, it was assumed that the enzyme is present in the final food; however, the Committee noted that the enzyme is expected to be absent from D-allulose product.

Evaluation

Negative results were observed with D-allulose in genotoxicity tests. A NOAEL of 1100 mg TOS/kg bw per day was identified, the highest dose tested, in a short-term (90-day) oral toxicity study in rats. When the dietary exposure estimate for the highest consumers (90th percentile for infants and children) of 0.38 mg TOS/kg bw per day was compared with the NOAEL of 1100 mg TOS/kg bw per day, an MOE of nearly 3000 was calculated. On this basis, the Committee established an ADI "not specified"[1] for D-allulose 3-epimerase from *A. globiformis* M30 expressed in *E. coli* K-12 W3110 when the enzyme is used in the applications specified, at the levels specified and in accordance with good manufacturing practice.

A toxicological monograph and a dietary exposure assessment were prepared.

A chemical and technical assessment and specifications were prepared.

References

1. Report of the Forty-eighth Session of the Codex Committee on Food Additives, Xi'an, China, 14–18 March 2016 (http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-711-48%252FReport%252FREP16_FAe.pdf, accessed 28 February 2020).

2. Evaluation of allergenicity of genetically modified foods. Report of a Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology 22–25 January 2001. Rome: Food and Agriculture Organization of the United Nations; Geneva: World Health Organization; 2001 (http://www.who.int/foodsafety/publications/gmo-allergenicity/en, accessed 28 February 2020).

3. Codex Alimentarius. Foods derived from modern biotechnology. Annex 1. Assessment of possible allergenicity. Rome: Food and Agriculture Organization of the United Nations; Geneva: World Health Organization; 2009 (http://www.fao.org/docrep/011/a1554e/a1554e00.htm).

[1] The reader is referred to Annex 2, reference 241, for clarification on the ADI "not specified".

3.1.3 Carbohydrate-derived fulvic acid

Explanation

The Committee was asked by the CCFA at its Fifty-first Session *(1)* to evaluate carbohydrate-derived fulvic acid (CHD-FA). The Committee has not previously evaluated this compound. In the CCFA request, the potential uses are described as follows: "CHD-FA liquid would be a suitable preservative for acidic foods such as jams, salad dressings, fruit and vegetable juices, pickles and carbonated drinks. Fulvate (CHD-FA powder) would be a suitable preservative in dry products, such as cereals, maize, soup powders and meal replacements."

A number of unpublished and published reports on absorption, distribution, excretion and toxicity in animals and on human tolerance were submitted by the sponsor. The Committee also conducted a literature search in Medline, Toxline, Scopus and SciFinder with the keywords "carbohydrate-derived fulvic acid", "clinical", "toxicology", "genotoxicity", "metabolism", "absorption", "excretion", "ADME". Two additional relevant publications were found.

Chemical and technical information was also provided.

Chemical and technical considerations

According to the sponsor, "carbohydrate-derived fulvic acid" is derived from non-catalytic wet oxidation of carbohydrate from sugar cane (sucrose, > 98% purity) with pure oxygen (> 99.5%) and purified water at high temperature (150–200 °C). The material is filtered through a 5000-Da filter to deliver a dark red-to-brown solution consisting of low-molecular-weight components. Alternatively, the material is filtered through a 400-Da filter to deliver a yellow solution consisting of lower-molecular-weight components. The required concentration is achieved through controlled evaporation. The 5000 Da-filtered dark red-to-brown and the 400 Da-filtered yellow solutions are CHD-FA products. In addition, there appears to be a third product that is dehydrated, which is unspecified, other than being described as "fulvate" in the submission.

The chemical and structural formulae and the formula weight provided are typical of fulvic acid, but the chemical name provided is not consistent with fulvic acid (CAS 479-66-3). The definition and explanation of the product do not provide clear information about the substance(s) intended to be used as a food additive.

The submitted assay for CHD-FA appears to be based on the principle of loss on drying, and the product is indicated to have a purity of 4.5–5.5%. The proposed method has insufficient specificity. No information on the other components of the product was provided.

The parameters proposed for evaluation of the identity and purity of the product are insufficient, and no method validation parameters were provided. Because of the lack of data, the product could not be characterized.

Biochemical aspects

A single oral dose (100 mg/kg bw) of ^{3}H-CHD-FA was administered to male rats, and blood was collected at various times over 1 week *(2)*. To estimate oral bioavailability, ^{3}H-CHD-FA (1 mg/kg bw) was injected intravenously for comparison with the data obtained after oral administration. Recovery of the administered doses was low, only about 79% being present after oral or intravenous dosing, indicating that appreciable tritium–hydrogen exchange had occurred to yield tritiated water, which was most likely excreted in breath and saliva. The oral bioavailability was calculated to be 78%. The authors reported that the half-life of ^{3}H-CHD-FA in plasma was shorter (119 h) after oral dosing than after intravenous dosing (178 h). A plausible explanation for this anomaly is that it was the disposition of tritiated water that was being measured, in addition to radiolabelled fulvic acid. This explanation would also be consistent with the observation that at least 80% of all radiolabelled metabolites in urine were excreted within 24 h of dosing, irrespective of the route of administration. The Committee concluded that the study was of limited value for this evaluation.

Toxicological studies

In a poorly reported 6-week, repeated-dose study in female rats only, treated by oral gavage with either 0 or 100 mg/kg bw per day of CHD-FA *(3)*, only a few parameters were assessed. The test material was also unclear, as it was described as "fulvic acid (potassium salt)" in one part of the study report and as "CHD-FA" elsewhere in the report. The Committee concluded that the study was of limited value for this evaluation.

A 6-month, repeated-dose study was conducted in female rats only, treated by oral gavage with either 0 or 100 mg/kg bw per day of test material *(4)*. As for the 6-week study, conducted in the same laboratory, the description of the test material was unclear. No adverse effects were reported, but very few parameters were assessed, and the reporting was very limited. The Committee noted that no rationale was given for using only female animals and a single dose level of 100 mg/kg bw per day.

A developmental toxicity study was conducted, in which groups of 10 pregnant rats were treated by gavage with 0 or 100 mg/kg bw per day of CHD-FA (not further specified) from 3 days before fertilization to gestation day 14 *(5)*. The dams were allowed to litter, and the pups were assessed at 2 weeks of age. The

CHD-FA-treated group had a smaller average litter size and significantly higher pup weights at birth. No other effects were reported.

The Committee noted that the designs of none of the above three studies *(3–5)* conformed to internationally recognized test guidelines and concluded that these limited studies were unsuitable for this evaluation.

In a combined repeated-dose study with the reproduction/developmental toxicity screening test (OECD TG 422) in rats *(6)*, CHD-FA (not further specified) was given orally by gavage at doses of 0 (vehicle control), 100, 300 and 1000 mg/kg bw per day. There were no signs of reproductive or developmental toxicity at doses ≤ 1000 mg/kg bw per day. The author stated that statistically significant increases in liver weight, both absolute and relative to terminal body weight, were observed in animals of both sexes treated with 1000 mg/kg bw per day and in female animals treated with 300 mg/kg bw per day. The Committee noted, however, that, at 300 mg/kg bw per day, a statistically significant increase in liver weight was also observed in male animals. Minimal to slight centrilobular hepatocyte hypertrophy was observed in four males treated with 1000 mg/kg bw per day and in one male receiving 300 mg/kg per day. Slight follicular cell hypertrophy was observed in the thyroid in two males treated with 1000 mg/kg bw per day, three males and three females treated with 300 mg/kg per day and three males treated with 100 mg/kg bw per day. The authors considered that the effects detected in the liver and thyroid were adaptive, noting that increases in liver weight associated with centrilobular hypertrophy often indicate adaptive induction of microsomal enzymes in response to administration of xenobiotics. Such enzymes (e.g. T4–UDP glucuronyl transferase) also metabolize thyroid hormones and thus trigger an increase in thyroid-stimulating hormone. The Committee noted that this mechanism is well known in rats and is usually considered not to be relevant to humans *(7)*. In some cases, however, thyroidal histopathological changes could be attributable to a direct-acting thyroid toxicant. Therefore, in the absence of thyroid hormone analyses in the study by Dunster *(6)*, it is unclear whether the association between the liver and thyroid findings is causal *(8)*. A study by Vucskits et al. *(9)*, in which fulvic acid in the diet of rats increased thyroid-stimulating hormone concentrations in a dose-related manner, did not resolve this issue.

Genotoxicity was evaluated in bacterial reverse mutation assays with plate incorporation and pre-incubation methods both with and without rat S9 metabolism for 400-Da and 5000-Da CHD-FA *(10,11)*, in an *in vitro* micronucleus test in human peripheral blood lymphocytes with CHD-FA (not further specified) and in an *in vivo* bone marrow micronucleus test in mice with 5000-Da CHD-FA administered by intraperitoneal injection *(12)*. All the results obtained were negative. The Committee noted, however, that the *in vitro* micronucleus test in peripheral blood human lymphocytes was unsuitable for evaluation. The major

shortcomings of the study included an inappropriate cytotoxicity method (cell viability by MTT) for selection of dose levels to be scored and use of a wrong concentration of cytochalasin-B (4.5 mg/mL). In addition, the incidence of micronucleated binucleated cells (16.8%) in the negative control was outside the normal range of values (0.3–1.2%) found for negative controls in human lymphocyte cultures, and the study was performed only in the absence of S9 metabolic activation. The Committee also noted that, while bacterial reverse mutation assays were performed with both the 400-Da and the 5000-Da forms of CHD-FA, the bone marrow micronucleus test was performed only with the 5000-Da form. The Committee also noted the sponsor's claim that the product has antimicrobial activity, which puts into question the suitability of the bacterial reverse mutation assay.

Assessment of dietary exposure

Both the sponsor and the Committee assessed dietary exposure, but, in the absence of adequate characterization of the articles of commerce intended for use and the inadequacy of the toxicological data supplied, dietary exposure was not considered further.

Evaluation

The Committee concluded that the available data are inadequate for an evaluation of the safety of CHD-FA. Further data, as indicated in the recommendations below, are necessary to complete the evaluation.

A toxicological monograph was not prepared.

The Committee assessed the chemical and technical information received and concluded that there was insufficient information to prepare specifications for CHD-FA.

Recommendations

The Committee requires data to characterize the products of commerce in order to evaluate the product for use as a preservative. The required information includes a detailed description of the manufacturing processes and thorough chemical characterization of the commercial products.

The following information is required:

- the full composition of the products;
- a detailed description of the manufacturing process;
- analytical methods and data on method validation; and
- analytical data for five non-consecutive batches of commercial products, including information on impurities.

The sponsor is encouraged to offer a rationale for whether a single monograph covering all products or individual monographs should be prepared.

Given the deficiencies of the toxicological database, the Committee recommends that the following studies be conducted. The test protocols should be in accordance with the relevant current guidelines, and the test materials should be well characterized in relation to the article(s) of commerce:

- absorption, distribution, metabolism and excretion (ADME);
- repeated-dose 90-day oral toxicity in rodents;
- two-generation reproductive toxicity or extended one-generation reproductive toxicity;
- prenatal developmental toxicity;
- additional studies, including an in vitro micronucleus test in mammalian cells, might be required, depending on elucidation of the article(s) of commerce and the provision of full information on their composition; and
- information on the potential of the material to induce antimicrobial resistance.

In addition, use levels should be provided for estimating dietary exposure.

References

1. Report of the Fifty-first Session of the Codex Committee on Food Additives, Jinan, China, 25–29 March 2019. Rome: Food and Agriculture Organization of the United Nations; Geneva: World Health Organization; 2019 (REP19/FA) (http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-711-51%252FReport%252FREP19_FAe.pdf).

2. Cnubben N, De Bie, A. Main study on the kinetics, absorption, distribution and excretion of ^{3}H-labelled CHD-FA following oral, dermal and intravenous dosing in male Sprague Dawley rats. Unpublished report submitted to WHO by PfeinsmitH SA (Pty) Ltd, Cape Town, South Africa; 2012 (Project Number: V 9305/02).

3. Medlen CE. Chronic toxicity study: A 6-week toxicity study was completed using 40 female old Sprague Dawley rats (6–8 weeks). Unpublished report. Pretoria: Department of Pharmacology, Faculty of Health Sciences, University of Pretoria; 2007.

4. Medlen CE. An investigation of the safety of a carbohydrate-derived fulvic acid (CHD-FA) administered topically and by gavage in rats. Unpublished report. Pretoria: Department of Pharmacology, Faculty of Health Sciences, University of Pretoria; 2014.

5. Sabi R, Very P, Jansen van Rensburg CE. Carbohydrate-derived fulvic acid (CHD-FA) inhibits carrageenan-Induced inflammation and enhances wound healing: efficacy and toxicity study in rats. Drug Dev Res. 2012;73:18–23.

6. Dunster J. CHD-FA: Oral gavage combined repeat dose toxicity study with reproduction/developmental toxicity screening test in the rat. Unpublished report submitted to WHO by PfeinsmitH SA (Pty) Ltd, Cape Town, South Africa (Project Number: 2814/0007); 2012.

7. Cappen CC. Mechanisms of chemical injury of thyroid gland. Prog Clin Biol Res. 1994;387:173–91.

8. Sutcliff C, Harvey PW. Endocrine disruption of thyroid function: chemicals, mechanisms, and toxicopathology. In: Darbre PD, editor. Endocrine disruption and human health. Amsterdam: Elsevier; 2015;201–17.

9. Vucskits AV, Hullar I, Bersenyi A, Andrasofszky E, Kulcsar E, Szabo J. Effect of fulvic and humic acids on performance, immune response and thyroid function in rats. J Anim Physiol Anim Nutr. 2010;94:721–8.

10. Bowles AJ, Thompson PW. CHD-FA 400: Reverse mutation assay "Ames test" using *Salmonella typhimurium* and *Escherichia coli*. Unpublished report submitted to WHO by PfeinsmitH SA (Pty) Ltd, Cape Town, South Africa (Project Number: 2814/0002); 2009.

11. Bowles AJ, Thompson PW. CHD-FA 5000: Reverse mutation assay "Ames test" using *Salmonella typhimurium* and *Escherichia coli*. Unpublished report submitted to WHO by PfeinsmitH SA (Pty), Cape Town, South Africa Ltd (Project Number: 2814/0003); 2010.

12. Gandy JJ, Medlen CE. The genotoxicity of filtered and unfiltered fulvic acid derived from a carbohydrate source (CHD-FA) using an in vitro micronucleus assay. Unpublished report. Pretoria: Department of Pharmacology, Faculty of Health Sciences, University of Pretoria; 2008.

3.1.4 Jagua (genipin-glycine) blue

Explanation

Jagua (genipin-glycine) blue, hereafter referred to as Jagua blue, is a water-soluble blue colorant. It is obtained by reacting genipin, present in the extract of unripe *Genipa americana* Linne (Rubiaceae) fruit, with stoichiometric equivalents of glycine. The main colouring component in Jagua blue is the genipin-glycine polymer (CAS No. 1314879-21-4). Additionally, low-molecular-weight components, specifically three genipin-glycine dimers, which are also blue, are present at low levels.

Jagua blue was previously evaluated by JECFA, at its 84th meeting, in 2017 (Annex 2, reference *234*). The NOAELs in two 90-day toxicological studies in rats and dogs were 330 and 338 mg/kg bw per day for the blue polymer, respectively, which were both the highest doses tested. On the basis of these NOAELs and a conservative exposure estimate of 11 mg/kg bw per day, the MOE for Jagua blue was approximately 30. In view of the limited biochemical and toxicological database and the low MOE, the Committee was unable to complete the evaluation of Jagua blue, and no ADI was established. The Committee was concerned about the potential toxicity of the low-molecular-weight fraction of the total colouring matter in Jagua blue and recommended that additional biochemical and toxicological information (e.g. studies of ADME, long-term

toxicity, carcinogenicity, reproductive and developmental toxicity) be made available, including with higher doses of the blue polymer and the dimers, in order to complete an evaluation of the safety of Jagua blue. For this, additional information was requested on: characterization of the low-molecular-weight components of the blue polymer, a validated method for the determination of dimers and data on the concentrations of dimers in five batches of the commercial product.

In response to these requests, the sponsor provided a dossier to the current Committee containing a new 12-month toxicological study with in-utero exposure of rats, an updated dietary exposure assessment and additional chemical and technical data. A literature search in PubMed and Embase for literature published between January 2017 and May 2020 with the search terms "Jagua blue", "genipin-glycine", "Genipa americana" or "Gardenia blue", which is a genipin–amino acid/peptide polymer with a structure similar to that of Jagua blue, identified five additional publications. None of the publications provided relevant information for the current evaluation.

Chemical and technical considerations

Genipa americana L. is a small to medium-sized tree *(1)* belonging to the Rubiaceae family and native to central and tropical South America *(2)*. The fruit of the plant is edible and is a popular source of beverages *(3)*. It is referred to as jagua fruit, as *chipara*, *guayatil*, *maluco*, *caruto* or *huito (4)* in Spanish and as genipap in English.

Jagua fruit contains high levels of a cyclopentan-[C]-pyranskeleton class of compounds called iridoids *(5,6)*. Three iridoids in this fruit have been reported (genipin, geniposidic acid and geniposide). Of these, genipin is present at the highest concentrations in unripe fruit *(7)* and is responsible for the bluish-purple colour formed upon exposure of the white pulp to air. Formation of the colour has been attributed to the ability of genipin to cross-link with primary amines present in amino acids and proteins, in the presence of oxygen, to produce water-soluble blue pigments *(8–14)*.

Jagua blue is obtained by reacting genipin in the filtered aqueous extract of the unripe fruit with stoichiometric amounts of glycine and heating at 70 °C for 2 h. When the reaction is complete, the product is centrifuged and concentrated and/or dried. The blue colour is due to both the polymer (average molecular weight, 6000 Da) that is composed of repeating dimers $(C_{27}H_{25}O_8N_2)_n$ and minor quantities of three dimers. The polymer and the dimers have been quantified and characterized by high-performance liquid chromatography, nuclear magnetic resonance spectroscopy (^1H, ^{13}C), infrared spectroscopy and mass spectroscopy. The molecular formulae of the three identified dimers are $C_{28}H_{28}N_2O_8$ (CAS No.

1313734-13-2), $C_{27}H_{25}N_2O_8$ (CAS No. 1313734-14-3) and $C_{27}H_{24}N_2O_8$ (CAS No. 104359-67-3).

The product of commerce is formulated in powder form and is obtained by concentrating, mixing with a food-grade carrier such as modified starch or maltodextrin, spray-drying and sieving. The product is standardized on the basis of genipin-glycine blue polymer. Unreacted genipin is not detectable in the final product. The product consists of a blue polymer (30–40%), sugars (25–30%), modified starch (20–22%), protein (approximately 7%) and water (approximately 5%). On the basis of a study with Jagua blue in solution at pH 3.6 in the dark for 50 days at 20 °C, the solution was predicted to have a half-life of 86–105 days *(15)*.

Biochemical aspects

The Committee at its 84th meeting concluded that the main colouring component of Jagua blue, the blue polymer, is unlikely to be absorbed intact from the gastrointestinal tract because of its high molecular weight (~ 6000 Da). This conclusion was supported by a study in which the blue polymer was not detectable in plasma of dogs given Jagua blue at doses up to 338 mg/kg bw per day for 90 days *(16)* and an *in vitro* study with the Caco-2 intestinal barrier model, which demonstrated that the blue polymer was poorly passively absorbed *(17)*. The 84th JECFA noted that some evidence, such as green urine observed in dogs given Jagua blue, suggested that a small proportion of the compound, "possibly the smallest coloured molecular species (such as genipin-glycine dimers or other coloured low molecular weight components), could be absorbed". In the newly available long-term toxicity study *(18)*, green urine was observed in a few Jagua blue-treated rats, primarily in the high-dose group. Although the presence of dimeric Jagua blue was not confirmed in any of the green urine samples, the possibility that dimers were absorbed and excreted could not be excluded. The Committee noted, however, that rats excreted green urine only sporadically and transiently and that its presence might also be explained by faecal contamination or an unclean periurethral area at the time of urine collection rather than by absorption of the coloured material. There was no indication of bioaccumulation.

Toxicological studies

The Committee at its 84th meeting discussed an oral gavage acute toxicity test in rats that showed no adverse effects at the highest tested dose of 660 mg/kg bw *(19)*. They also discussed two short-term studies, in rats and in dogs. When Jagua blue was administered by gavage to rats for 90 days, no deaths or treatment-related toxicological effects were reported. The NOAEL was identified as 330 mg/kg bw per day on a blue-polymer basis, the highest dose tested *(20)*. In the second study, Jagua blue was administered to beagle dogs by gavage for 90 days. No deaths or

treatment-related toxicological effects were reported. The NOAEL was identified as the highest dose tested, 338 mg/kg per day on a blue-polymer basis *(16)*.

A 12-month dietary study in rats with in-utero exposure that was compliant with good laboratory practice *(18)* was available to the Committee at the current meeting. A Jagua blue product was administered at 0, 2500 (low), 12 500 (medium) or 50 000 (high) mg/kg feed to rats from before mating until weaning in the parental generation and up to about 52 weeks in the F1 generation. The test article contained 36.4% of the blue polymer and 0.11–0.26% of the dimers. No adverse or biologically relevant treatment-related effects were reported in a comprehensive set of observations, including target organ toxicity, a functional observation battery of tests, reproductive indices and developmental outcomes at concentrations up to 50 000 mg/kg feed. Discoloured faeces, attributed to the colour of the test item, were observed in all treated animals. Gross pathology showed blue discoloration of the kidneys in most F0 animals in the high-dose group but not in F1 animals. Blue discoloration in the gastrointestinal segments was reported in five F1 animals but not in F0 animals. As there was no evidence of histopathological changes associated with these macroscopic findings, the Committee did not consider the tissue discoloration to be toxicologically relevant.

This study was conducted with a longer exposure time and higher doses of Jagua blue than in the short-term studies in rats and dogs previously evaluated by the Committee at its 84th meeting, as recommended. Overall, the Committee concluded that this long-term toxicity study demonstrated lack of chronic toxicity and reproductive and developmental toxicity after prenatal and 1 year of exposure to Jagua blue at concentrations up to 50 000 mg/kg in feed. The NOAEL was identified as 50 000 mg/kg feed, the highest concentration tested, equal to a dose of 3095 and 5634 mg/kg bw per day for F0 males and females, respectively, and 3385 and 3750 mg/kg bw per day in F1 males and females, respectively, expressed as Jagua blue. The Committee chose the lowest body weight-based NOAEL, 3095 mg/kg bw per day, as the basis for establishing an ADI. This NOAEL corresponds to 1127 mg/kg bw per day on a blue-polymer basis.

Because of inadequate characterization of the test article and the relatively low doses administered to the animals in the short-term studies available at the 84th meeting, the previous Committee raised concern about the composition and potential toxicity of the low-molecular-weight fraction of the total colouring matter in Jagua blue. At the current meeting, chemical characterization of the dimers indicated that the three genipin–glycine dimers accounted for only 0.11–0.26% of the test article used in the long-term study.

At its 84th meeting, three genotoxicity studies on Jagua blue were available to the Committee: a bacterial reverse mutation assay, an *in vitro* mouse lymphoma assay and an *in vivo* mammalian micronucleus induction assay. No genotoxicity potential of Jagua blue was identified. No new genotoxicity studies with Jagua

blue were available to the current meeting. In the absence of genotoxicity and the lack of treatment-related neoplastic or preneoplastic histopathological lesions in rats exposed in utero and for 1 year postnatally in the new study, the current Committee concluded that a 2-year carcinogenicity study was not required.

Assessment of dietary exposure

The Committee reviewed estimates of dietary exposure to Jagua blue on a blue-polymer basis from the Chronic Individual Food Consumption database summary statistics (CIFOCOss) and the European Food Safety Authority (EFSA) Comprehensive European Food Consumption Database and proposed maximum use levels for 22 food categories. The sponsor updated the proposed maximum use levels from those provided at the 84th JECFA meeting. A fixed fraction (40%) was applied to estimates of use of Jagua blue in order to derive the corresponding exposure levels on a blue-polymer basis. As estimated from the with the CIFOCOss and EFSA databases, the mean and high (95th percentile) dietary exposure of infants and toddlers (≤ 3 years) to Jagua blue on a blue-polymer basis were 0.3–8.3 mg/kg bw per day and 2.7–11.5 mg/kg bw per day, respectively.

In addition, dietary exposure estimates prepared by the sponsor based on food consumption data from national surveys in Brazil, the United Kingdom and the USA and adjusted use levels were available to the Committee. The adjusted use levels were derived by applying factors for the assumptions that only a portion of a food category or food would be expected to be coloured (e.g. coated sweets) or a seasonal adjustment factor (e.g. flavoured milk) to the proposed maximum use levels. The highest 95th percentile exposure to Jagua blue on a blue-polymer basis based on adjusted use levels was 2.3 mg/kg bw per day for children aged 2–5 years in the USA. This estimate is lower than those based on the CIFOCOss and EFSA databases and proposed maximum use levels but was not considered by the Committee, which noted that it was not clear how well the adjusted levels reflect actual use levels in foods available on the market, as Jagua blue has not yet been marketed globally.

The Committee concluded that the conservative estimates of the range of high-level exposure, 2.7– 11.5 mg/kg bw per day for infants and toddlers (≤ 3 years), should be considered in the safety assessment of Jagua blue on a blue-polymer basis.

Evaluation

The Committee considered that the new toxicological data and additional characterization of the test compound provided adequate information for completing the safety evaluation of Jagua blue. The new 12-month study of rats

exposed in utero *(18)* was conducted for a longer exposure time and at higher doses of Jagua blue, as recommended by the Committee at its 84th meeting. Although no new toxicokinetics study was available, newly developed analytical methods for the dimers provided acceptable characterization of the test article, thus reducing the uncertainty of the safety assessment due to limited biochemical (ADME) information.

An ADI of 0–11 mg/kg bw was established by the Committee for Jagua blue, on a blue-polymer basis. This ADI was based on the absence of treatment-related long-term toxicity and of reproductive and developmental toxicity in the 12-month rat dietary study with in-utero exposure, in which the NOAEL was identified as 1127 mg/kg bw per day of the blue polymer, the highest dose tested. The ADI was established by applying an uncertainty factor of 100 to the NOAEL to allow for inter- and intra-species differences.

The Committee noted that the upper end of the high-level dietary exposure estimate for Jagua blue, on a blue-polymer basis, for infants and toddlers of 11.5 mg/kg bw per day is in the region of the upper bound of the ADI. In view of the conservative nature of the dietary exposure assessments, in which it was assumed that all foods contained Jagua blue on a blue-polymer basis at the maximum use level, and because the ADI was based on a NOAEL that was the highest dose tested, the Committee concluded that the estimated dietary exposure to Jagua blue, on a blue-polymer basis, does not represent a health concern.

A toxicological monograph and an addendum to the dietary exposure assessment were prepared.

The Committee revised specifications for Jagua blue and withdrew the tentative status. The chemical and technical assessment was updated.

References

1. World Investment Report 2005. Geneva: United Nations Conference on Trade and Development; 2005.
2. Ono M, Ishimatsu N, Masuoka C, Yoshimitsu H, Tsuchihashi R, Okawa M, et al. Three new monoterpenoids from the fruit of Genipa americana. Chem Pharm Bull. 2007;55(4):632–4.
3. Ueda S, Iwahashi Y, Tokuda H. Production of anti-tumor-promoting iridoid glucosides in *Genipa americana* and its cell cultures. J Nat Prod. 1990;54(6):1677–80.
4. Ramos-de-la-Peña AM, Montañez JC, de la Luz Reyes-Vega M, Hendrickx ME, Contreras-Esquivel JC. Recovery of genipin from genipap fruit by high pressure processing. LWT-Food Sci Technol. 2015;63(2):1347–50.
5. Dinda B, Debnath S, Harigaya Y. Naturally occurring iridoids. A review. Part 1. Chem Pharmaceut Bull. 2007;55(2):159–222.
6. Dinda B, Debnath S, Harigaya Y. Naturally occurring secoiridoids and bioactivity of naturally occurring iridoids and secoiridoids. A review, part 2. Chem Pharmaceut Bull. 2007;55(5):689–728.

7. Neri-Numa IA, Gabriel-Pessôa M, Silvano Arruda H, Araujo Pereira G, NIcolau Paulino B, Figueiredo Angolini CF, et al. Genipap (*Genipa americana* L.) fruit extract as a source of antioxidant and antiproliferative iridoids. Food Res Int. 2020;134:109–252.

8. Touyama R, Takeda Y, Inoue K, Kawamura I, Yatsuzuka M, Ikumoto T, et al. Studies on the blue pigments produced from genipin and methylamine. I. Structures of the brownish-red pigments, intermediates leading to the blue pigments. Chem Pharmaceut Bull. 1994;42(3):668–73.

9. Touyama R, Inoue K, Takeda Y, Yatsuzuka M, Ikumoto T, Moritome N, et al. Studies on the blue pigments produced from genipin and methylamine. II. On the formation mechanisms of brownish-red intermediates leading to the blue pigment formation. Chem Pharmaceut Bull. 1994;42(8):1571–8.

10. Fujikawa S, Fukui Y, Koga K, Kumada J. Brilliant skyblue pigment formation from gardenia fruits. J Ferment Technol. 1987;65:419–24.

11. Paik YS, Lee CM, Cho MH, Hahn TR. Physical stability of the blue pigments formed from geniposide of gardenia fruits: effects of pH, temperature, and light. J Agric Food Chem. 2001;49:430–2.

12. Park JE, Lee JY, Kim HG, Hahn TR, Paik YS. Isolation and characterization of water-soluble intermediates of blue pigments transformed from geniposide of *Gardenia jasminoides*. Agric Food Chem. 2002;50:6511–4.

13. Cho YJ, Kim SY, Kim J, Choe EK, Kim SI, Shin HJ. One-step enzymatic synthesis of blue pigments from geniposide for fabric dyeing. Biotechnol Bioprocess Eng. 2006;11:230–4.

14. Lee JH, Lee DU, Jeong CS. Gardenia jasminoides Ellis ethanol extract and its constituents reduce the risks of gastritis and reverse gastric lesions in rats. Food Chem Toxicol. 2009;47:1127–31.

15. Brauch JE, Zapata-Porras SP, Buchweitz M, Aschoff JK, Carle R. Jagua blue derived from *Genipa americana* L. fruit: a natural alternative to commonly used blue food colorants? Food Res Int. 2016;89(1):391–8.

16. Mancari F. Jagua extract: 90-day oral toxicity study in the dog. Study no. 2015-0175. Unpublished report by Accelera SRL, Nerviano, Milan, Italy; 2016. Sponsored by AnaPath GmbH, on behalf of Ecoflora. Submitted to WHO by Ecoflora SAS, Colombia.

17. Gilbert J. Caco-2 permeability testing of Jagua extract. Study No. CYP1291-R1. Unpublished report by Cryprotex US, LLC, Watertown (MA), USA; 2015. Prepared for Ricerca Biosciences, Concord (OH), USA. Submitted to WHO by Ecoflora SAS, Colombia.

18. Takawale P, Hamann U, Häuselmann N, Weise K, Weber K, Hellenbrand T. 12 Months repeated dose toxicity study Including in-utero exposure in Wistar rats with Jagua extract. Study No. 171414. Unpublished report by BSL Bioservice, Planegg, Germany; 2019. Submitted to WHO by Ecoflora SAS, Colombia.

19. Allingham P. Acute oral toxicity (fixed dose procedure) limit test with jagua extract. Report no. 136354. Unpublished report by BSL Bioservice, Planegg, Germany, 2014. Submitted to WHO at the 84th JECFA meeting by Ecoflora SAS, Colombia.

20. Allingham P, Weise K, Weber K, Janku S. 90-Day repeated dose oral toxicity study in Wistar rats with jagua extract including a 28-day recovery period. Report no. 132041. Unpublished report by BSL Bioservice, Planegg, Germany, 2014. Submitted to WHO by Ecoflora SAS, Colombia.

3.1.5 Lipase from *Mucor javanicus*

Explanation

At the request of the CCFA at its Forty-ninth Session *(1)*, the Committee evaluated the safety of lipase (triacylglycerol lipase; Enzyme Commission No. 3.1.1.3) from *Mucor javanicus* (also known as *M. circinelloides*), which has not previously been evaluated by the Committee. Lipase from animal tissues (forestomach of calves, caprine kids and lambs or animal pancreatic tissue) was evaluated at its 15th meeting (Annex 2, reference *26*), and an ADI "not limited"[2] was allocated. Besides its use as a processing aid in cheese-making and modification of lipids, lipase from animal tissues is also used as a flavour enhancer (INS 1104). The Committee evaluated lipase from *Aspergillus oryzae* var. at its 18th meeting and established an ADI "not specified" (Annex 2, reference *35*). At its 55th meeting, however, the Committee removed the tentative specifications for this enzyme preparation because the requested data had not been submitted (Annex 2, reference *149*). The consequences of the withdrawal of specifications for lipase from *A. oryzae* var. on its ADI were not addressed during that meeting. Lipase from *Fusarium heterosporum* expressed in *Ogatae polymorpha* was evaluated by the Committee at its 80th meeting, which established an ADI "not specified" for its use in the manufacture of bakery products, pasta and noodles, in egg yolk and in the degumming of edible oil (Annex 2, reference *82*).

In this report, the expression "lipase" refers to the lipase enzyme and its amino acid sequence, the expression "liquid enzyme concentrate" refers to the test material used in the toxicity studies evaluated, and the expression "enzyme preparation" refers to the product formulated for commercial use.

The Committee at the present meeting considered the submitted data and conducted a literature search in the PubMed database with the linked search terms "lipase" and "mucor" or "javanicus". The search yielded 188 references, but no relevant published studies on biochemical or toxicological aspects were found.

Genetic background

The production organism, *M. javanicus*, is a filamentous fungus often found in soil, plants and decaying fruits and vegetables. The taxonomy of *Mucor* species was confirmed by its macroscopic and microscopic characteristics. The *M. javanicus* production strain was further verified as a strain of *M. javanicus* by phylogenetic analysis of the internal transcribed spacer rDNA sequence from the results of a homology search with BLAST. *Mucor* species are recognized for their use in food applications *(2)*, including as source organisms in the production of lipase used in food processing *(3)*. *M. javanicus* is considered an occasional

[2] The term ADI "not limited" is no longer used by JECFA. It has the same meaning as ADI "not specified". The reader is referred to Annex 2, reference 241, for clarification of ADI "not specified".

opportunistic human pathogen and has been known rarely to cause acute and rapidly developing mucormycoses in susceptible populations, including those with acidotic diabetes, malnourished children, severely burnt patients, patients undergoing immunosuppressive therapy and patients with leukaemia, lymphoma or AIDS *(4,5)*. As no viable organisms are present in the enzyme preparation, however, the Committee concluded that there is no concern regarding the potential human pathogenicity of the production strain *M. javanicus*.

The *M. javanicus* production strain was obtained by chemical mutagenesis followed by selection of individual colonies of the parent strain, *M. javanicus* IAM 6018. The parent strain was originally housed at the Institute of Applied Microbiology Culture Collection; it is presently held at the Japan Collection of Microorganisms under *M. javanicus* JCM 22477.

Chemical and technical considerations

The lipase is produced by controlled aerobic batch fermentation of a pure culture of a selected strain of *M. javanicus* (AE-LM). The enzyme is secreted into the fermentation broth and is separated from the biomass by a series of filtration steps. The filtrate containing the enzyme is further concentrated. Filtration is performed at various stages throughout the recovery process to control against microbial contamination. The liquid enzyme concentrate is spray-dried with dextrin to produce the powdered enzyme preparation. The entire process is performed in accordance with current good manufacturing practice with food-grade raw materials. The final lipase enzyme preparation is free of the production strain and other insoluble substances. The enzyme preparation conforms to the General Specifications and Considerations for Enzyme Preparations Used in Food Processing (Annex 2, reference *185*).

Lipase catalyses the hydrolysis of mono-, di- and triglycerides containing short-, medium- and long-chain fatty acid moieties. It is intended for use in the processing of food ingredients containing naturally occurring fats and oils, including flour, cheese, egg whites and flavourings of vegetable, animal or microbiological origin. The use of lipase in food processing increases the fatty acid content for the purpose of improving the organoleptic (flavour and texture) and/or physical properties (consistency and texture) of the ingredients or final food. Lipase activity is measured in units per gram (U/g) based on the release of fatty acids from a triglyceride-containing substrate. One unit is defined as the amount of enzyme that liberates 1 μmol of free fatty acid from an olive oil substrate per minute under the assay conditions. The mean activity of lipase from three batches of the enzyme concentrate was 31 100 U/g.

The mean TOS content of the enzyme concentrate is 410 mg/g. The TOS include the enzyme of interest and residues of organic materials, such as proteins,

peptides and carbohydrates, derived from the production organism during the manufacturing process. Lipase enzyme preparation is used at concentrations up to 1357 mg TOS/kg raw material, depending on the proposed food application. Lipase is heat-denatured and inactivated during processing at high temperatures (> 60 °C), such that the enzyme will have no technological effect in the final food.

Biotransformation

No information was available.

Assessment of potential allergenicity

Lipase from *M. javanicus* was evaluated for potential allergenicity with the bioinformatics criteria recommended by FAO/WHO *(6,7)* and modified by the Committee at its 80th meeting (Annex 2, reference *223*). The amino acid sequence of lipase from *M. javanicus* was compared with the amino acid sequences of known allergens in publicly available databases. A search for matches with > 35% identity in a sliding window of 80 amino acids, a search for sequence identity of eight contiguous amino acids and a full-length FASTA sequence search produced no matches. Therefore, the Committee considered that dietary exposure to lipase from *M. javanicus* is not anticipated to pose a risk of allergenicity.

Toxicological data

In a 13-week study of oral toxicity in rats, no treatment-related adverse effects were seen when the lipase liquid enzyme concentrate was administered by gavage at doses up to 800 mg TOS/kg bw per day, the highest dose tested *(8)*. The lipase liquid enzyme concentrate was not genotoxic in a bacterial reverse mutation assay *(9)* or in an *in vitro* chromosomal aberration assay *(10)*. The Committee had no concern with respect to the genotoxicity of the lipase enzyme preparation.

Assessment of dietary exposure

The Committee evaluated two estimates of dietary exposure, the first submitted by the sponsor based on exposure to two lipase enzymes and one estimated by the Committee. The estimate of dietary exposure calculated by the Committee for lipase from *M. javanicus* was deemed appropriate for use in the evaluation. The estimate was derived with the budget method and was based on maximum use levels of 27.1 mg TOS/kg for solid foods and 20 mg TOS/kg for non-milk beverages and an assumption that 25% of the food supply would contain the enzyme preparation. The theoretical maximum daily intake was estimated to be 0.84 mg TOS/kg bw per day.

Evaluation

Negative results were obtained in genotoxicity tests, and no treatment-related adverse effects were seen at the highest dose tested (800 mg TOS/kg bw per day) in a 13-week study of oral toxicity in rats. A comparison of the estimated dietary exposure of 0.84 mg TOS/kg bw per day with the highest dose tested of 800 mg TOS/kg bw per day gives an MOE of at least 900. On this basis the Committee established an ADI "not specified"[3] for the lipase enzyme preparation from *M. javanicus*, used in the applications specified and in accordance with good manufacturing practice.

A toxicological monograph and a dietary exposure assessment were prepared.

New specifications and a chemical and technical assessment were prepared.

References

1. Report of the Forty-eighth Session of the Codex Committee on Food Additives, Xi'an, China, 14–18 March 2016 (REP16/FA). Rome: Food and Agriculture Organization of the United Nations; and Geneva: World Health Organization, Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2016 (http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-711-48%252FReport%252FREP16_FAe.pdf).

2. Bourdichon F, Casaregola S, Farrokh C, Frisvad JC, Gerds ML, Hammes WP, et al. Food fermentations: microorganisms with technological beneficial use. Int J Food Microbiol. 2012;154(3):87–97.

3. Pariza MW, Johnson EA. Evaluating the safety of microbial enzyme preparations used in food processing: update for a new century. Regul Toxicol Pharmacol. 2001;33(2):173–86.

4. Rippon JW. Chapter 23. Aspergillosis. In: Medical mycology, third edition. Philadelphia (PA): WB Saunders Co.; 1988:618–50.

5. Ellis DH. Clinical mycology: the human opportunistic mycoses. Underdale: Gillingham Printers; 1994.

6. Evaluation of allergenicity of genetically modified foods. Report of a Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology 22–25 January 2001. Rome: Food and Agriculture Organization of the United Nations; Geneva: World Health Organization; 2001 (http://www.who.int/foodsafety/publications/gmo-allergenicity/en, accessed 28 February 2020).

7. Codex Alimentarius. Foods derived from modern biotechnology, second edition. Rome: Food and Agriculture Organization of the United Nations; Geneva: World Health Organization; 2009 (http://www.fao.org/3/a-a1554e.pdf).

8. Okazaki S. A 13-week oral gavage toxicity study of lipase concentrate in rats [in Japanese, English translation]. Unpublished study (No. B-7655) from Gotemba Laboratory, Bozo Research Center Inc., Gotemba-shi, Japan, 2014. Submitted to WHO by Amano Enzyme Inc., Nagoya, Japan.

[3] The reader is referred to Annex 1, reference 241, for clarification of the term "ADI not specified".

9. Oguma Y. A bacterial reverse mutation test of triacylglycerol lipase. [in Japanese, English translation]. Unpublished report (Study No. T-1725) from Tokyo Laboratory, Bozo Research Center Inc., Tokyo, Japan, 2015. Submitted to WHO by Amano Enzyme Inc., Nagoya, Japan.

10. Fugiwara S. Chromosome aberration test of lipase cong. in cultured Chinese hamster (CHL/IU) cells [in Japanese, English translation]. Unpublished report (Study No. T-G162) from Tokyo Laboratory, Bozo Research Center Inc., Tokyo, Japan, 2015. Submitted to WHO by Amano Enzyme Inc., Nagoya, Japan.

3.1.6 Phosphatidylinositol-specific phospholipase C expressed in *Pseudomonas fluorescens*

Explanation

At the request of the CCFA at its Forty-eighth Session *(1)*, the Committee evaluated the safety of phosphatidylinositol-specific phospholipase C (1-phosphatidyl-1D-*myo*-inositol-4,5-bisphosphate inositoltrisphospho-hydrolase, Enzyme Commission No. 3.1.4.11) expressed in *Pseudomonas fluorescens*. The Committee has not previously evaluated this enzyme preparation. The Committee evaluated phospholipase C expressed in *Pichia pastoris*[4] at its 69th meeting and established an ADI "not specified" for its use in refining vegetable oils intended for human consumption (Annex 2, reference *191*).

In this monograph, the expression "phosphatidylinositol-specific phospholipase C" (PI-PLC) refers to the PI-PLC enzyme and its amino acid sequence; the expression "liquid enzyme concentrate" refers to the test material used in the toxicity studies evaluated; and the expression "enzyme preparation" refers to the preparation formulated for commercial use.

The Committee at its present meeting considered the submitted data and conducted a literature search in the PubMed database with the linked search terms "phospholipase" and "pseudomonas" and "fluorescens". This yielded 39 references, but no relevant published studies on biochemical or toxicological aspects were found.

Genetic background

P. fluorescens is a common Gram-negative, aerobic, saprophytic, rod-shaped bacterium, widely distributed in the environment *(3)*. It can be found in soils, water, plants, animals, the hospital environment and human clinical specimens and is a normal inhabitant of the plant rhizosphere or phyllosphere *(4)*. It can grow on a range of organic substrates and can remain viable for long periods in a wide variety of habitats. Strains of *Pseudomonas* are ubiquitous saprophytes, with

[4] In 2019, EFSA issued a scientific opinion on phospholipase C, updating the name of the microorganism from *Pichia pastoris* to *Komagataella phaffii (1)*.

low virulence *(5)*. *P. fluorescens* is a non-pathogenic microorganism with a long history of safe use in food.

The *P. fluorescens* production strain BD27719 was prepared by transforming the *P. fluorescens* recipient strain with an expression plasmid carrying a synthetic, codon-optimized modified PI-PLC gene for thermal stability and a *pyrF* gene selective marker. The donor organism of the parent PI-PLC is unknown; however, the amino acid sequence of the PI-PLC has > 95% similarity to phospholipases from *Bacillus* species. The *P. fluorescens* recipient strain was obtained from the parent *P. fluorescens* MB101 (ATCC PTA-7841) by deletion of the orotidine-5'-phosphate decarboxylase gene (*pyrF*), followed by introduction of the *lacI* gene from *Escherichia coli* K-12 (ATCC 4707). The expression plasmid was transformed to carry the modified PI-PLC gene under regulation of the *tac* promoter and was transferred into the recipient *P. fluorescens* strain. The insertion of the modified PI-PLC gene and the absence of antibiotic resistance genes in the *P. fluorescens* production strain were confirmed by DNA sequence analysis. The stability of the expression plasmid was confirmed by quantitative polymerase chain reaction and restriction digestion. The mobilization potential and transferability of the expression plasmid were characterized and confirmed to be poor.

The sponsor submitted three studies with *P. fluorescens* in mice *(6–8)*. As no viable production organism is expected to be present in the enzyme preparation, these studies were not considered in the evaluation.

Chemical and technical considerations

PI-PLC is produced by controlled submerged aerobic fed-batch fermentation of a pure culture of the *P. fluorescens* production strain. During fermentation, isopropyl β-D-thiogalactopyranoside (IPTG) is added to induce enzyme production. The enzyme is recovered from the fermentation broth after a heat lysis step and separated from the cell debris by filtration. The supernatant containing the enzyme is filtered, followed by concentration and stabilization. The liquid enzyme concentrate, free of the production strain, is formulated into a liquid enzyme preparation by the addition of glycerol. The entire process is performed in accordance with current good manufacturing practice with raw materials of food-grade quality. The final enzyme preparation is not expected to contain any major food allergens from the fermentation medium. Additionally, IPTG is present in the final enzyme preparation at a maximum level of 400 µg/g of enzyme preparation. The enzyme preparation conforms to the General Specifications for Enzyme Preparations used in Food Processing (Annex 2, reference *185*).

PI-PLC catalyses the hydrolysis of phosphatidylinositol to inositol monophosphate and diacylglycerol. It is intended for use as a processing aid in

refining edible vegetable oils containing phospholipids to improve their stability and quality. Phosphatidylinositol negatively affects the taste, colour and stability of vegetable oil, while the hydrolytic products do not. The enzyme can be combined as a liquid preparation with two other phospholipases (phospholipase C from *Pichia pastoris* and phospholipase A2 from *Aspergillus niger*) to promote conversion of phospholipids found in crude vegetable oils. The enzyme's activity is determined spectrophotometrically; the assay quantifies the hydrolysis of 4-methylumbelliferyl *myo*-inositol-1-phosphate substrate by PI-PLC and the liberation of 4-methylumbelliferone at 380 nm after a 5-min reaction. Enzyme activity is expressed in inositol phosphate releasing units (IPRUs). One IPRU is the quantity of enzyme that liberates 1 μmol of 4-methylumbelliferone from 4-methylumbelliferyl myo-inositol-1-phosphate per minute at pH 7.5 and 37 °C. The mean activity of PI-PLC from three batches of enzyme concentrate was 15 128 IPRUs/g.

The mean TOS content of the enzyme concentrate is 143 mg/g. TOS include the enzyme of interest and residues of organic materials, such as proteins, peptides and carbohydrates derived from the production organism during the manufacturing process. The enzyme preparation is intended for use as a processing aid at a maximum level of 15 μg TOS per gram of edible vegetable oil. PI-PLC is expected to be removed from the oil during processing, and any remaining enzyme will be inactivated. On the basis of this level, the maximum content of IPTG from the enzyme preparation in processed vegetable oil amounts to 120 μg/kg of oil. IPTG is expected to be removed with the water phase during degumming of vegetable oil.

Biotransformation

On the basis of *in vitro* digestibility studies with simulated gastric fluid and simulated intestinal fluid, it can be expected that the enzyme is fully degraded in the gastrointestinal tract *(9)*.

Assessment of potential allergenicity

PI-PLC from a genetically modified strain of *P. fluorescens* was evaluated for allergenicity by the bioinformatics criteria recommended by FAO/WHO *(10,11)*, modified by the Committee at its 80th meeting (Annex 2, reference *223*). The amino acid sequence of the enzyme was compared with the amino acid sequences of known allergens in a publicly available database. A search for matches with > 35% identity in a sliding window of 80 amino acids, a search for sequence identity of eight contiguous amino acids and a full-length FASTA sequence search produced no matches. Therefore, the Committee considered that dietary exposure to PI-PLC from a genetically modified strain of *P. fluorescens* would

not pose a risk of allergenicity. This consideration is further supported by the finding in the *in vitro* digestibility study that the enzyme is fully degraded in the gastrointestinal tract.

Toxicological data

In a 13-week study of oral toxicity in rats, no treatment-related adverse effects were seen when the PI-PLC liquid enzyme concentrate was administered by gavage at doses up to 1871 mg TOS/kg bw per day, the highest dose tested *(12)*. The PI-PLC enzyme concentrate was not genotoxic in a bacterial reverse mutation assay *(13)* or in an *in vitro* chromosomal aberration assay *(14)*. The Committee had no concern with respect to the genotoxicity of the PI-PLC enzyme preparation.

Assessment of dietary exposure

Two estimates of dietary exposure were available, one from the sponsor and the other calculated by the Committee. Both estimates were based on per capita consumption data (derived from food balance sheets) for plant-based (vegetable) oils in regional and in cluster diets, respectively. The maximum use level of 0.015 mg TOS/g fat or oil was used in the calculations. The estimated dietary exposures to IP-PLC ranged from 0.003 to 0.01 mg TOS/kg bw per day. The Committee noted that the enzyme would be removed during processing of the oil and that any remaining enzyme would become denatured and inactive; therefore, no or negligible dietary exposure to the enzyme would be expected from the final food. In addition, the Committee noted that dietary exposure to IPTG used in the manufacturing process of the enzyme would not be a safety concern.[5]

Evaluation

Negative results were obtained in genotoxicity tests, and no treatment-related adverse effects were seen with PI-PLC enzyme concentrate at the highest dose tested (1871 mg TOS/kg bw per day) in the 13-week study of oral toxicity in rats. A comparison of the highest estimated dietary exposure of 0.01 mg TOS/kg bw per day with the highest dose tested of 1871 mg TOS/kg bw per day gives an MOE of at least 187 100. On this basis, the Committee established an ADI "not specified"[6] for the PI-PLC enzyme preparation expressed in *P. fluorescens*, used in the applications specified and in accordance with good manufacturing practice.

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[5] Even if it is assumed that the concentration in vegetable oil is 120 µg/kg, the dietary exposure would be at least 10 times lower than the threshold of toxicological concern of 90 µg/day for IPTG (Cramer class III; according to ToxTree version 3.1.0.1851, there were no structural alerts for genotoxicity). This exposure estimate is based on the highest reported per capita consumption of "plant origin fat" of 59.7 g/person per day from the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) cluster diets *(15)*.

[6] The reader is referred to Annex 1, reference *241*, for clarification of an ADI "not specified".

A toxicological monograph and a dietary exposure assessment were prepared.

New specifications and a chemical and technical assessment were prepared.

References

1. Report of the Forty-eighth Session of the Codex Committee on Food Additives, Xi'an, China, 14–18 March 2016. Rome: Food and Agriculture Organization of the United Nations; Geneva: World Health Organization, Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2016 (REP16/FA; http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-711-48%252FReport%252FREP16_FAe.pdf).
2. CEP Panel. Scientific opinion on the safety evaluation of the food enzyme phospholipase C from a genetically modified *Komagataella phaffii* (strain PRF). EFSA J. 2019;17(4):5682.
3. Bradbury JF. Guide to plant pathogenic bacteria. Kew (Richmond): International Mycological Institute; 1986.
4. Consensus document on information used in the assessment of environmental applications involving *Pseudomonas* (Series on harmonization of regulatory oversight in biotechnology No. 6). Paris: Organization for Economic Co-operation and Development; 1997 (http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=OCDE/GD(97)22&docLanguage=En).
5. Palleroni N. Human and animal pathogenic Pseudomonas. In: Balows A, Truper HG, Dworkin M, Harder W, Schleifer KH, editors. The prokarytoes, Vol. III. 2nd edition. Berlin: Springer-Verlag; 1992:3086–103.
6. George SE, Nelson GM, Kohan MJ, Brooks LR, Boyd C. Colonization and clearance of environmental microbial agents upon intranasal exposure of strain C3H/HeJ mice. J Toxicol Environ Health A. 1999;56(6):419–31.
7. George SE, Nelson GM, Boyd C, Kohan MJ, Brooks LR. Survival of environmental microbial agents in CD-1 mice following oral exposure. Microbiol Ecol Health Dis. 2000;12(2):92–8.
8. Landry TD, Chew L, Davis JW, Frawley N, Foley HH, Stelman SJ, et al. Safety evaluation of an α-amylase enzyme preparation derived from the archaeal order Thermococcales as expressed in *Pseudomonas fluorescens* biovar I. Regul Toxicol Pharmacol. 2003;37(1):149–68.
9. De Bruine A, Farres-Mendez M, Den Hartog J. Digestibility of PI-PLC under simulated gastric and intestinal conditions. Unpublished report, 2019. Submitted to WHO by DSM Food Specialties, Delft, The Netherlands.
10. Evaluation of allergenicity of genetically modified foods. Report of a Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology 22–25 January 2001. Rome: Food and Agriculture Organization of the United Nations; Geneva: World Health Organization; 2001 (http://www.who.int/foodsafety/publications/gmo-allergenicity/en, accessed 28 February 2020).
11. Codex Alimentarius. Foods derived from modern biotechnology, second edition. Rome: Food and Agriculture Organization of the United Nations; Geneva: World Health Organization; 2009 (http://www.fao.org/3/a-a1554e.pdf).
12. Sathish PM. Repeated dose (90-day) oral toxicity study by gavage with enzyme preparation of *Pseudomonas fluorescens* containing Pi-PLC activity in Wistar rats. Unpublished study (Study number

G8635) from Advinus Therapeutics Ltd, Bangalore, India, 2013. Submitted to WHO by DSM Food Specialties, Delft, The Netherlands.

13. Verspeek-Rip CM. Evaluation of mutagenic activity of enzyme preparation of *Pseudomonas fluorescens* containing Pi-PLC activity in the *Salmonella typhimurium* reverse mutation assay and the Escherichia coli reverse mutation assay (with independent repeat). Unpublished report (Project number 502549) from WIL Research Europe B.V., 's-Hertogenbosch, The Netherlands, 2013. Submitted to WHO by DSM Food Specialties, Delft, The Netherlands.

14. Buskens CAF. Evaluation of the ability of enzyme preparation of *Pseudomonas fluorescens* containing Pi-PLC activity to induce chromosome aberrations in cultured peripheral human lymphocytes (with repeat experiment). Unpublished report (Project number 502550) from WIL Research Europe BV, 's-Hertogenbosch, The Netherlands, 2013. Submitted to WHO by DSM Food Specialties, Delft, The Netherlands.

15. GEMS/Food Cluster Diets 2012 [online database]. Geneva: World Health Organization, Global Environment Monitoring System, Food Contamination Monitoring and Assessment Programme; 2012 (https://www.who.int/foodsafety/databases/en).

3.1.7 Riboflavin from *Ashbya gossypii*

Riboflavin from *Ashbya gossypii* was on the agenda of the current meeting at the request of the Codex Committee on Food Additives at its fifty-first session *(1)* for assessment of its safety and dietary exposure and for preparation of new specifications. Because of time constraints, the assessments of safety and dietary exposure were not completed.

Riboflavin or 7,8-dimethyl-10-(1´-D-ribityl)isoalloxazine (IUPAC name: 7,8-dimethyl-10-[(2S,3S,4*R*)-2,3,4,5-tetrahydroxypentyl]benzo[*g*]pteridine-2,4-dione; CAS No. 83-88-5), commonly known as vitamin B_2, was last evaluated for specifications by JECFA as a synthetic product in 1987 and as a product of fermentation from a genetically modified strain of *Bacillus subtilis* in 1999 (Annex 2, reference *185*).

The Committee at its present meeting drafted a chemical and technical assessment and new specifications for riboflavin from *A. gossypii* from the data submitted by the sponsor, but they were not finalized for publication. The Committee recognized the benefits of simultaneous review and harmonization of new specifications with existing specifications for riboflavin as a synthetic product and as a product of *B. subtilis*. The Committee recommends that this work be undertaken at a future meeting.

Reference

1. Report of the Fifty-first Session of the Codex Committee on Food Additives, Jinan, China, 25–29 March 2019. Rome: Food and Agriculture Organization of the United Nations; Geneva: World Health Organization; 2019 (REP19/FA) (http://www.fao.org/fao-who-codexalimentarius/sh-proxy/

en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-711-51%252FReport%252FREP19_FAe.pdf).

3.2 Exposure assessment

3.2.1 Sucrose esters of fatty acids (INS 473) and sucrose oligoesters types I and II (INS 473a)

Explanation

Sucrose esters of fatty acids (INS 473) (SEFs) and sucrose oligoesters (SOEs) types I and II (INS 473a) are used mainly as emulsifiers and stabilizers in food. SEFs are authorized for use in 50 food categories at maximum permitted levels (MPLs) ranging from 200 mg/kg up to 20 000 mg/kg, as specified in the Codex General Standard for Food Additives (GSFA) *(1)*. SOEs types I and II are authorized for use in all but seven of the same food categories at the same MPLs. The MPLs for SEFs and SOEs in the same food categories are valid for single use of the two food additives or in combination, together with sucroglycerides (INS 474).

SEFs and SOEs were on the agenda of the present meeting of the Committee at the request of the Fifty-first Codex Committee on Food Additives *(2)*. Only an evaluation of the exposure was requested. Dietary exposure to SEFs has not previously been evaluated by the Committee, whereas dietary exposure of the populations of Japan and the USA to SOEs was evaluated in 2010 at the 71st meeting of the Committee (Annex 2, reference *196*). During that meeting, dietary exposure to SOEs was calculated from typical and maximum use levels and mean food consumption amounts derived from national nutrition surveys for 11 food categories. The mean dietary exposure estimates based on typical use levels were 1.9 mg/kg bw per day for Japan and 2.5 mg/kg bw per day for the USA. The corresponding estimates based on maximum use levels were 3.7 and 4.6 mg/kg bw per day. The Committee concluded that these were overestimates, because not all foods in each food category will contain SOEs and consumers will not consistently select those foods containing the additives. At its 71st meeting, the Committee also estimated dietary exposure to SOEs on the basis of a maximum use level of 5000 mg/kg for all food categories in which SOEs are authorized in the USA combined with mean food consumption data for that country. The mean dietary exposure was 0.8 mg/kg bw per day, and the 90th percentile dietary exposure was 1.6 mg/kg bw per day.

Assessment of dietary exposure

For the current meeting, the sponsor provided the highest technical use levels for the sum of SEFs and SOEs for 46 food categories in the GSFA. The Committee did not use the budget method *(3–5)* to calculate a theoretical high level of

dietary exposure to the sum of SEFs and SOEs with the use levels provided by the sponsor, because more refined dietary estimates of exposure to the sum of the two food additives were available from the sponsor and in the literature. In addition, the Committee did not assess international dietary exposure with the use levels provided and the commodity-based food consumption data of the GEMS/Food database cluster diets, the FAO/WHO CIFOCOss database or individual food consumption from the FAO/WHO Global Individual Food consumption data Tool (GIFT). The main reasons were that:

- many of the recorded foods in these databases were raw food commodities, whereas food additives are added to processed foods only; and
- the highest technical use levels were provided for broad food categories, which would have resulted in highly conservative estimates of dietary exposure.

The Committee evaluated the estimates of dietary exposure to the sum of SEFs and SOEs submitted by the sponsor for Japan and estimates for Europe *(6)* and the USA *(7)*. The estimates are listed in Table 1. The sponsor also provided poundage data for the sum of SEFs and SOEs for Japan. These data were not considered, because more refined estimates were available.

Dietary exposure to the sum of SEFs and SOEs in Japan was estimated from individual food consumption data collected in 2005–2007 combined with MPLs from the GSFA and the highest reported technical use levels. Only the mean dietary exposure was estimated. The mean and high estimated dietary exposures for the European population and the population of the USA were based on individual food consumption data combined with (maximum) use levels for SEFs only. As the MPLs in the GSFA refer to both single and combined use of SEFs and SOEs, the dietary exposure estimates of SEFs for Europe and the USA were also considered to represent dietary exposure to the sum of the two food additives.

All the dietary exposure estimates listed in Table 1 are overestimates of the actual mean or high exposure to the sum of SEFs and SOEs in Europe, Japan and the USA. The main reason was the assumption that all foods that could contain the food additives did in fact contain SEFs and/or SOEs, whereas other food additives with the same functions in foods are available. SEFs and SOEs constituted 15% of all food additives used mainly as emulsifiers in Japan in 2013. At its 71st meeting, the Committee noted that SOEs are expected to account for ≤ 10% of the total market share of emulsifiers (Annex 2, reference *196*). In addition,

Table 1
Estimated dietary exposure to the sum of sucrose esters of fatty acids and sucrose oligoesters, types I and II

	Dietary exposure (mg/kg bw per day)[a]			
	Children[b]		General population[c]	
Country and levels	Mean	High	Mean	High
Japan				
MPLs	65[b]	–	23	-
Use level[d]	25	–	9	-
Europe				
Use level[e]				
Brand-loyal scenario	3.0–54.6	11.1–124.3	0.2–54.6	0.8–124.3
Non-brand loyal scenario	1.7–42.7	6.3–112.6	0.1–42.7	0.4–112.6
USA				
Maximum use level[e]		–	9	18

Sources: Sponsor and references *5* and *6*
[a] High dietary exposure: 95th percentile for Europe and 90th percentile for the USA
[b] Children aged 1–6 years in Japan and 1–9 years in Europe.
[c] The general population of Europe comprises infants (12 weeks–11 months), toddlers (12–35 months), children (3–9 years), adolescents (10–17 years), adults (18–64 years) and older adults (≥ 65 years); the general population of the USA covers the ages of 2–99 years. The age range of the general population of Japan was not specified.
[d] Use levels of sucrose esters of fatty acids and sucrose oligoesters, types I and II combined
[e] Use levels of sucrose esters of fatty acids.

the EFSA noted that data from Mintel's Global New Products Database[7] showed that SEFs were listed on the labels of only 0.2% of the foods in food categories in which the use of SEFs is authorized *(5)*. These foods were or had been available on the European market between January 2012 and October 2017. Another factor that contributed to the overestimates was the assumption that when SEFs or SOEs are used, they are always present at the reported (maximum) use levels.

The Committee considered that the high (95th percentile) dietary exposure of 113 mg/kg bw per day for children aged 3–9 years, in the non-brand loyal scenario, was the most suitable for evaluation of dietary exposure to the sum of SEFs and SOEs. The non-brand loyal estimate was selected because SEFs and SOEs are predominantly used as emulsifiers or stabilizers in foods for which other food additives with the same functions in foods are available, and SEFs and SOEs are not expected to influence the taste or appearance of foods.

Evaluation

At its 49th meeting, the Committee established a group ADI of 0–30 mg/kg bw for SEFs and sucroglycerides on the basis of their potential to induce laxative

[7] The Mintel Global New Products Database is an online database for monitoring new introductions of packaged goods into the market worldwide.

effects in adult volunteers at doses > 30 mg/kg bw per day, without applying an uncertainty factor (Annex 2, reference *131*). At its 71st meeting, the Committee noted that some of the components of SEFs may be present in significant amounts in SOEs and established a group ADI of 0–30 mg/kg bw for SEFs, SOEs and sucroglycerides (Annex 2, reference *196*).

The high dietary exposure estimate of the sum of SEFs and SOEs of 113 mg/kg bw per day for children aged 3–9 years exceeds the group ADI of 0–30 mg/kg bw per day by a factor of about 4. The Committee also noted that the dietary exposure estimates for some other age groups also exceeded the ADI.

The Committee noted that the high dietary exposure estimates are conservative, predominantly due to the assumptions that

- all foods that could contain SOEs and SEFs do in fact contain these food additives, whereas other food additives with the same functions in foods are available; and
- when SEFs or SOEs are used, they are always present at the reported use levels.

Therefore, the Committee considered that more refined dietary exposure estimates should be provided.

Recommendations

To refine the dietary exposure estimates of SEFs and SOEs, either alone or summed, the Committee recommends that sponsors submit information on:

- typical or mean and high use levels for foods in which the food additives are used; and
- foods (or food categories) in which the use of SEFs and/or SOEs is permitted but in which they are never used.

In both cases, the information should be as specific as possible, and the foods should be classified according to the FoodEx2 classification system, which is that used for the CIFOCOss and GIFT food consumption databases, or another appropriate system.

The Committee did not use the CIFOCOss and GIFT databases to assess dietary exposure to SEFs and SOEs, partly because calculations of exposure would have been laborious in view of the number of broad food categories for which use levels were provided. In order to use these data for dietary exposure assessment of food additives that are present in large numbers of food categories, a table should be developed to map the foods recorded in both databases according to the FoodEx2 classification to the food categories of the GSFA. That will also ensure that mapping is consistent for all meetings.

The Committee recommends that more detailed information on the use of SEFs and SOEs in foods and a mapping table be made available within 2 years.

A dietary exposure assessment was prepared.

References

1. Report of the Fifty-first Session of the Codex Committee on Food Additives, Jinan, China, 25–29 March 2019 (REP19/FA). Rome: Food and Agriculture Organization of the United Nations; (http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-711-51%252FReport%252FREP19_FAe.pdf).
2. Codex General Standard for Food Additives [online, food additives database]. Rome: Food and Agriculture Organization of the United Nations; 2019 (http://www.fao.org/gsfaonline/additives/index.html; accessed 6 May 2020).
3. Hansen SC. Acceptable daily intake of food additives and ceiling on levels of use. Food Cosmet Toxicol. 1966;4:427–32.
4. Hansen SC. Conditions for use of food additives based on a budget method for an acceptable daily intake. J Food Protect. 1979;42:429–34.
5. Principles and methods for the risk assessment of chemicals in food. Chapter 6. Dietary exposure assessment of chemicals in food. Geneva: World Health Organization; 2009 (https://apps.who.int/iris/bitstream/handle/10665/44065/WHO_EHC_240_9_eng_Chapter6.pdf;jsessionid=4C1B9C27E95AC6E3D7E82941C3B4F14C?sequence=9).
6. Scientific opinion on the refined exposure assessment of sucrose esters of fatty acids (E 473) from its use as a food additive. EFSA J. 2018;16(1);5087.
7. Shah R, Kolanos R, DiNovi MJ, Mattia A, Kaneko KJ. Dietary exposures for the safety assessment of seven emulsifiers commonly added to foods in the United States and implications for safety. Food Addit Contamin Part A. 2017;34:905–17.

3.3 Revision of specifications and analytical methods

3.3.1 Magnesium stearate (INS 470(iii))

Magnesium stearate (INS 470(iii)) was on the agenda of the present meeting at the request of the Codex Commission on Food Additives at its Fifty-first meeting for revision of the method of assay for determination of magnesium.

The Committee at its 80th meeting prepared new specifications for magnesium stearate and included an inductively coupled plasma–atomic emission spectrometric (ICP-AES) method for the assay of magnesium. The sponsor requested that this method be replaced by a titrimetic method, which is non-specific. The Committee at its current meeting assessed the information submitted and considered that there is no reason to limit the specifications to use of either ICP-AES or any other method. As there are equivalent methods for the

assay of magnesium, the Committee replaced the reference to ICP-AES with "use a method appropriate to the specified level".

3.3.2 Polyvinyl alcohol

Polyvinyl alcohol (INS No. 1203, PVOH) was on the agenda of the current meeting at the request of the CCFA at its Fifty-first Session *(1)* to revise the specifications for its solubility. The specifications of PVOH were last revised by JECFA at its 68th meeting, in 2007 (Annex 2, reference *187*), when the specification for solubility was set as "sparingly soluble in ethanol" on the basis of the information available at that time.

The Committee at its current meeting reviewed the information and data received to support a change of solubility to "practically insoluble or insoluble in ethanol" based on use of the solubility test by the JECFA method and an alternative method. The Committee took note of the relevant statement of EFSA *(2)* and of the tests performed by the Joint Research Center in support of changing the solubility criteria *(3)*. After reviewing the information, the Committee changed the solubility criteria to "practically insoluble or insoluble in ethanol".

The Committee noted that PVOH is produced by hydrolysis of polyvinyl acetate and that its solubility depends on the degree of hydrolysis and of polymerization. Both the hydrolysis range and the viscosity range for this food-grade PVOH are specified within narrow limits, and all products within these limits shall have the same solubility characteristics. The Committee noted, however, that different partially hydrolysed PVOH products with a viscosity as high as 120 mPa × s (4% solution at 20 °C) are available in commerce. The Committee recommended that the CCFA determine whether the food-grade PVOH products currently available in commerce comply with the narrow range of viscosity (4.8–5.8 mPa × s) and degree of hydrolysis (86.5–89%) in the specifications. Any deviations would necessitate a review of its safety evaluation.

The Committee also noted that the gas chromatographic method for determining methanol and methyl acetate in PVOH is a packed-column method and recommended that it be replaced by a suitable capillary or wide-bore column gas chromatographic method.

References

1. Report of the Fifty-first Session of the Codex Committee on Food Additives, Jinan, China, 25–29 March 2019. Rome: Food and Agriculture Organization of the United Nations; Geneva: World Health Organization; 2019 (REP19/FA) (http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-711-51%252FReport%252FREP19_FAe.pdf).

2. European Food Safety Authority. Statement on the request for a modification of the specification on solubility of the food additive polyvinyl alcohol (E 1203) in ethanol and its possible impact on the safety assessment. EFSA J. 2014;12(9):3820.

3. Lopes JFA, Simoneau C. Solubility of polyvinyl alcohol in ethanol (EFSA supporting publication 2014:EN-660); Parma: European Food Safety Authority; 2014.

3.3.3 Sorbitan esters of fatty acids (INS 491, INS 492 and INS 495)

Sorbitan monostearate (INS 491), sorbitan tristearate (INS 492) and sorbitan monopalmitate (INS 495) were on the agenda of the current meeting at the request of the CCFA at its Fifty-first Session *(1)* to replace the specification for congealing range. The Committee noted that the method for identifying the congealing range reported in the JECFA monographs for INS 491, 492 and 495 is empirical and difficult to perform (Annex 2, reference *63*), and the results are not repeatable. Determination of the congealing range was included as an identification method because it is correlated with the type and content of fatty acids (sorbitan monostearate vs sorbitan tristearate) and their length (sorbitan monopalmitate vs sorbitan monostearate). The congealing range also depends, however, on variations in the content of minor constituents, such as that of stearic acid in edible palmitic acid, a raw material for the manufacture of sorbitan monopalmitate. The Committee considered that a specification for the fatty acid components (a minimum–maximum range) and additional compositional parameters would be more appropriate.

The Committee noted that toxicological evaluations of the above additives were often performed with samples for which the congealing range was not reported. JECFA established a group ADI of 0–25 mg/kg bw for sorbitan monostearate (INS 491), sorbitan tristearate (INS 492), sorbitan monopalmitate (INS 495), sorbitan monolaurate (INS 493) and sorbitan monooleate (INS 494) at its 26th meeting in 1982 (Annex 2, reference *59*). Sorbitan monolaurate and sorbitan monooleate were not on the agenda of the present meeting because their specifications do not include a congealing range. The Committee noted that the group ADI is not expressed based on sorbitan content, although the sorbitan content of the five esters varies widely.

JECFA established the specifications for sorbitan esters of fatty acids at its 17th, 33rd and 39th meetings of (Annex 2, references *32*, *83* and *101*, respectively). The specifications in those early evaluations, such as for solubility and impurities, should be revised, the manufacturing information updated and the ADI re-evaluated. The Committee recommends that a new call for data be issued in order to proceed with an updated safety evaluation and specifications for the five sorbitan esters of fatty acids at the same time.

The Committee also noted that five polyoxyethylene sorbitan esters (polysorbates) were evaluated by JECFA at its 17th meeting (Annex 2, reference *32*), and specifications were established. The Committee recommends that a new call for data be issued for their full evaluation.

In view of these recommendations and because of the limited time available at the current meeting, the agenda of the meeting was changed, and the specifications for sorbitan esters of fatty acids were not revised.

Reference

1. Report of the Fifty-first Session of the Codex Committee on Food Additives, Jinan, China, 25–29 March 2019 (REP19/FA). Rome: Food and Agriculture Organization of the United Nations; (http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-711-51%252FReport%252FREP19_FAe.pdf).

4. Flavouring agents

The Committee evaluated 13 flavouring agents for the first time and re-evaluated 14 agents. Full specifications were prepared for 12 of the agents, and tentative specifications were prepared for three, as the safety evaluations for these flavouring agents were not completed. Information on the safety evaluations and specifications is summarized in Annex 3. Details of further toxicological studies and other information required for certain substances are summarized in section 5.

4.1 Safety evaluations

4.1.1 Amino acids and related substances

Introduction

The Committee evaluated 20 members of this group of flavouring agents at its 63rd meeting (Annex 2, reference *173*) and six members of the group at its 76th meeting (Annex 2, reference *211*). The Committee concluded that none of the 26 flavouring agents was a safety concern at the estimated dietary exposures.

The Committee evaluated an additional six flavouring agents in the group of amino acids and related substances for the first time. In addition, the Committee considered new data on 20 previously evaluated flavouring agents in this group and data on L-cystine, a structurally related substance. The additional flavouring agents in this group are betaine (No. 2265), *N*-acetyl-glutamate (No. 2269), L-cysteine methyl ester hydrochloride (No. 2270), two dipeptides, glutamyl-2-aminobutyric acid and glutamyl-norvaline (Nos 2266 and 2268), and one tripeptide, glutamyl-norvalyl-glycine (No. 2267). Three of the flavouring agents in this group (Nos 2266–2268) are reported to be flavour modifiers. Betaine (No. 2265) has been reported to occur naturally in sugar beet molasses, wheat, mushroom, seafood and red and white wines *(1–5)*.

The Committee noted at its 63rd meeting (Annex 2, reference *173*) that amino acids may react with other food constituents upon heating. The mixtures formed are commonly referred to as "process flavours". The Committee has not reviewed the safety of process flavours. The safety evaluation of the flavouring agents in this group of amino acids and related substances is therefore conducted on the basis that these flavouring agents are present in an unchanged form at the time of consumption.

The six additional members of this group were evaluated according to the Revised Procedure for the Safety Evaluation of Flavouring Agents (Annex 2, reference *230*).

Assessment of dietary exposure

The total annual volume of production of the six additional flavouring agents in the group of amino acids and related substances is 11 000 kg in the USA and 420 kg in Latin America *(6,7)*. More than 99% of the annual production volume in the USA and 62% in Latin America is accounted for by betaine (No. 2265).

Dietary exposures were estimated with both the single portion exposure technique (SPET) and the maximized survey-derived intake (MSDI) method; the higher of the two values for each flavouring agent is reported in Table 2. The SPET and MSDI method values ranged from 1800 to 300 000 µg/day and 0.01 to 1142 µg/day, respectively. The estimated daily dietary exposure was highest for betaine (No. 2265) (300 000 µg/day, SPET value for non-alcoholic soft beverages).

Absorption, distribution, metabolism and elimination

Information on the ADME of flavouring agents in the group of amino acids and related substances is provided in monographs from the 63rd and 76th meetings (Annex 2, references *174* and *212*). Additional information on the ADME of three new flavouring agents (Nos 2266, 2267 and 2268) evaluated at this meeting and additional studies on a previously reviewed material (No. 1435) have become available.

The amino acids and related substances used as flavouring agents include amino acids, modified L-amino acids that are endogenous (e.g. *N*-acetylglutamate, No. 2269) or foreign to the human body (exogenous) (e.g. L-cysteine methyl ester hydrochloride, No. 2270) and dipeptides (e.g. glutamyl-norvaline, No. 2268) and tripeptides (e.g. glutamyl-norvalyl-glycine, No. 2267). The most frequent initial metabolic reaction for modified amino acids is hydrolysis to generate the parent amino acid(s), which usually occurs in either the stomach or the small intestine. Amino acids are absorbed readily through the intestinal mucosa and distributed via the blood; their entry into mammalian cells is facilitated by multiple amino acid transport systems. D-Amino acid stereoisomers and those L-amino acids that are not required for protein synthesis undergo catabolism, primarily in the liver. Amino acids are not stored in the liver in humans but are catabolized by oxidative deamination, in which amino acids are deaminated to yield α-ketoacids, which are either completely oxidized to CO_2 and water or provide three or four carbon units that are converted via gluconeogenesis to glucose or undergo ketogenesis to yield ketone bodies.

Application of the Revised Procedure for the Safety Evaluation of Flavouring Agents

Step 1. There are no structural alerts for genotoxicity for the additional six flavouring agents (Nos 2265–2270) in this group. Chemical-specific genotoxicity

Table 2

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

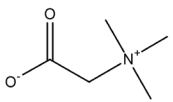
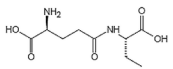
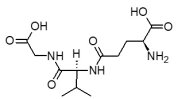
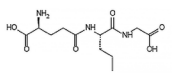
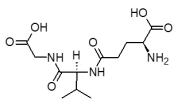
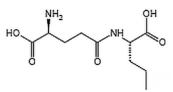
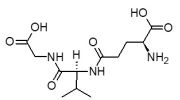
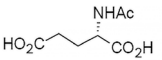
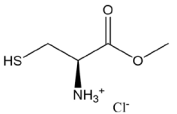
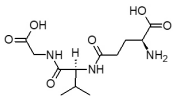
Flavouring agent	No.	CAS No. and structure	Step 4 Does intake exceed the threshold of toxicological concern?[e]	Step 5 Does a NOAEL exist for the flavouring agent or a structural relative that provides an adequate MOE?	Comments on predicted metabolism	Structural relative name (No.) and structure	Conclusion based on current estimated dietary exposure
Structural class I							
Betaine	2265	107-43-7 	Yes: SPET: 300 000	Yes. The NOAEL of 1428 mg/kg bw per day for betaine in a 28-day feeding study in rats *(10)* is at least 285 times the estimated daily dietary exposure to No. 2265 when used as a flavouring agent.	Note 1		No safety concern
Glutamyl-2-aminobutyric acid	2266	16869-42-4 	Yes; SPET: 6000	Yes. The NOAEL of 1000 mg/kg bw per day for the structurally related substance glutamyl-valyl-glycine (No. 2123) in a 28-day feeding study in rats *(11)* is at least 10 000 times the estimated daily dietary exposure to No. 2266 when used as a flavouring agent.	Note 2	Glutamyl-valyl-glycine (No. 2123) 	No safety concern
Glutamyl-norvalyl-glycine	2267	16869-42-4 	Yes; SPET: 1 800	Yes. The NOAEL of 1000 mg/kg bw per day for the structurally related substance glutamyl-valyl-glycine (No. 2123) in a 28-day feeding study in rats *(11)* is at least 33 300 times the estimated daily dietary exposure to No. 2267 when used as a flavouring agent.	Note 2	Glutamyl-valyl-glycine (No. 2123) 	No safety concern
Glutamyl-norvaline	2268	71133-09-0 	Yes; SPET: 2 100	Yes. The NOAEL of 1000 mg/kg bw per day for the structurally related substance glutamyl-valyl-glycine (No. 2123) in a 28-day feeding study in rats *(11)* is at least 28 500 times the estimated daily dietary exposure to No. 2268 when used as a flavouring agent.	Note 2	Glutamyl-valyl-glycine (No. 2123) 	No safety concern

Table 2 (continued)

Flavouring agent	No.	CAS No. and structure	Step 4 Does intake exceed the threshold of toxicological concern?[e]	Step 5 Does a NOAEL exist for the flavouring agent or a structural relative that provides an adequate MOE?	Comments on predicted metabolism	Structural relative name (No.) and structure	Conclusion based on current estimated dietary exposure
N-Acetyl glutamate	2269	1188-37-0 	Yes; SPET: 15 000	Yes. The NOAEL of 914 mg/kg bw per day in a 28-day feeding study in rats *(12)* is at least 3600 times the estimated daily dietary exposure to No. 2269 when used as a flavouring agent.	Note 3		No safety concern
Structural class III							
L-Cysteine methyl ester hydrochloride	2270	18598-63-5 	Yes, SPET: 2 000	Yes. The NOAEL of 600 mg/kg bw per day for the structurally related substance L-cystine in a 93-day gavage study in rats *(13)* is at least 18 000 times the estimated daily dietary exposure to No. 2270 when used as a flavouring agent.	Note 2	Glutamyl-valyl-glycine (No. 2123) 	No safety concern

[a] Twenty-six flavouring agents in this group were previously reviewed by the Committee, at its 63rd and 76th meetings (Annex 2, references *173* and *211*).
[b] Step 1: Genotoxicity data for the newly evaluated flavouring agents in this group do not indicate potential genotoxicity.
[c] Step 2: Five of the additional flavouring agents are in structural class I (Nos 2265, 2266, 2267, 2268 and 2269), and one (No. 2270) is in structural class III.
[d] Step 3: Dietary exposures were estimated by both the SPET and the MSDI method, and the higher of the two values for each flavouring agent is reported in the table. The SPET gave the highest estimate for each flavouring agent. All dietary intake values are expressed in µg/day.
[e] Step 4: The threshold of toxicological concern for structural classes I and III are 1800 and 90 µg/day, respectively.

Notes:

1. Betaine is expected to be transformed by demethylation via a series of enzyme reactions that occur mainly in the mitochondria of liver and kidney cells. A methyl group is transferred from betaine to homocysteine to form dimethylglycine and methionine, respectively. Dimethylglycine is then further demethylated. Through this transmethylation process, betaine metabolism is connected to the homeostasis of methionine and homocysteine.
2. Hydrolysed to constituent amino acids
3. *N*-Acetyl-glutamic acid is an intermediate in the urea cycle, which is integral in the removal of excess nitrogen from humans. It is endogenous to humans and specifically present in the hepatic mitochondria. *N*-Acetyl-glutamate allosterically activates carbamoyl phosphate synthetase I, which is the first step of the urea cycle.

data on previously evaluated flavouring agents in this group and on the newly added agents do not indicate that they have genotoxic potential.

Step 2. In applying the Revised Procedure to the above-mentioned flavouring agents, the Committee assigned five agents (Nos. 2265–2269) to structural class I and one (No. 2270) to structural class III *(8)*.

Step 3. Dietary exposures were estimated with both the MSDI method and the SPET.

Step 4. The highest estimated dietary exposures for the five flavouring agents in structural class I are above the threshold of toxicological concern for the class (i.e. 1800 μg/person per day). The highest estimated dietary exposure of the one flavouring agent in structural class III is above the threshold of toxicological concern for the class (i.e. 90 μg/person per day). Evaluation of these flavouring agents therefore proceeded to Step 5.

Step 5. For betaine (No. 2256), the NOAEL of 1428 mg/kg bw per day in a 28-day dietary study in male and female rats *(9)* provides an adequate MOE (285) relative to the SPET estimate of 300 000 μg/day.

For glutamyl-2-aminobutryic acid (No. 2266), the NOAEL of 1000 mg/kg bw per day in a 28-day dietary study in male and female rats for the structurally related substance glutamyl-valyl-glycine (No. 2123) *(10)* provides an adequate MOE (10 000) in relation to the SPET estimate of 6000 μg/day. This NOAEL is appropriate for the structurally related flavouring agents glutamyl-norvalyl-glycine (No. 2267) and glutamyl-norvaline (No. 2268), as they are di- and tripeptide derivatives and are expected to follow the same metabolic pathways. For these structurally related flavouring agents, the NOAEL of 1000 mg/kg bw per day provides MOEs of 33 300 for glutamyl-norvalyl-glycine (No. 2267) and 28 500 for glutamyl-norvaline (No. 2268) relative to their SPET estimates of 1800 and 2100 μg/day, respectively.

For *N*-acetyl-glutamate (No. 2269), the NOAEL of 914 mg/kg bw per day in a 28-day dietary study in rats *(11)* provides an adequate MOE (3600) relative to its SPET estimate of 15 000 μg/day.

For L-cysteine methyl ester hydrochloride (No. 2270), the NOAEL of 600 mg/kg bw per day for the structurally related substance L-cystine in a 93-day gavage study in rats *(12)* provides an adequate MOE (18 000) relative to its SPET estimate of 2000 μg/day.

Table 2 summarizes the evaluations of the six flavouring agents in the group of amino acids and related substances that were considered at the present meeting (Nos 2265, 2266, 2267, 2268, 2269 and 2270).

Consideration of combined intakes from use as flavouring agents

The Committee previously considered the potential combined intake of this group of amino acids and related substances and identified no safety concern. Five of the additional flavouring agents in this group (Nos 2266, 2267, 2268, 2269 and 2270) have low MSDI values (0.01–0.04 μg/day) and would therefore make a negligible contribution to the combined intake of this group. Exposure to betaine (No. 2265) from its use as a flavouring agent, 1142 μg/day, is not significant in comparison with its intake from other dietary sources; for example, 100 g of spinach contain > 600 mg betaine *(5)*.

Consideration of additional data on previously evaluated flavouring agents

The Committee considered additional data on 20 of the 26 previously evaluated flavouring agents in this group. Studies of ADME (No. 1435), of short-term toxicity (Nos 1419–1424, 1426, 1428–1430, 1434, 1435, 1437–1439, 2119, 2120 and 2123), of long-term toxicity (Nos 1420 and 1422), of reproductive and developmental toxicity (Nos 1420, 1422, 1424 and 1435) and of genotoxicity (Nos 1420, 1421, 1424, 1427–1431, 1434, 1438, 2120 and 2123) were available. These data support the conclusions of the previous evaluations.

Conclusion

Studies of ADME, acute toxicity, short-term and long-term toxicity and genotoxicity were available for the 26 substances in this group of amino acids and related substances that were evaluated previously (Annex 2, references *174* and *212*). None raised safety concerns.

Studies of ADME (Nos 2266–2268), of short-term toxicity (Nos 2265 and 2269) and of genotoxicity (Nos 2265 and 2269) were available for the six additional flavouring agents, and a short-term toxicity study was available on L-cystine, a structurally related substance.

At its present meeting, the Committee concluded that the six additional flavouring agents (Nos 2265–2270) would not give rise to safety concerns at the current estimated dietary exposures.

The Committee also concluded that the additional data presented in this addendum do not give rise to safety concerns and further support the safety of the 26 previously evaluated flavours in this group.

An addendum to the monograph was prepared.

References

1. Meyers SP. Aquaculture feeds and chemoattractants. Infofish Market Dig. 1987;1/87:35–7.

2. Waggle DH, Lambert MA, Miller GD, Farrell EP, Devoe CW. Extensive analysis of flours and millfeeds made from nine different wheat mixes. Cereal Chem. 1967;44:48–60.

3. Clarke WC, Virtanen E., Blackburn J, Higgs DA. Effects of dietary betaine/amino acid additive on growth and seawater adaptation in yearling chinook salmon. Aquaculture. 1994;121:137–45.

4. Mar MH, Zeisel SH. Betaine in wine: answer to the French paradox? Med Hypotheses. 1999;53:383-5.

5. Zeisel SH, Mar MH, Howe JC, Holden JM. Concentrations of choline-containing compounds and betaine in common foods. J Nutr. 2003;133:1302–7.

6. 2015 IOFI Global Poundage Survey. International Organization of the Flavor Industry, Brussels, Belgium; 2018. Submitted to WHO by the International Organization of the Flavor Industry, Brussels, Belgium.

7. Interim inquiry on volume use and added use levels for flavoring agents to be presented at the JECFA 89th meeting. Private communication to the International Organization of the Flavor Industry Brussels, Belgium, 2019. Submitted to WHO by the International Organization of the Flavor Industry, Brussels, Belgium.

8. Cramer GM, Ford RA, Hall RL. Estimation of toxic hazard – a decision tree approach. Food Cosmet Toxicol. 1978;16(3):255–76.

9. Hayes KC, Pronczuk A, Cook MW, Robbins MC. Betaine in sub-acute and sub-chronic rat studies. Food Chem Toxicol. 2003;41:1685–700.

10. Okamura T. A 28-day dietary toxicity study of L-γ-glutamyl-L-valyl-glycine (KF001) in rats. Study no. B-6880. Gotemba Laboratory, Bozo Research Center Inc., Shizuoka, Japan. Unpublished report to the Expert Panel of the Flavor and Extract Manufacturers Association. Submitted to WHO by the International Organization of the Flavor Industry, Brussels, Belgium; 2010.

11. Harper MS, Shen ZA, Barnett JF Jr, Krsmanovic L, Myhre A, Delaney B. *N*-Acetyl-glutamic acid: evaluation of acute and 28-day repeated dose oral toxicity and genotoxicity. Food Chem Toxicol. 2009;47:2723–9.

12. Kawai Y, Okai M, Takeshita S, Tomita M, Nakagawa H, Yoshinaka T, et al. Acute and subacute toxicity of cystenosine, inosine and cystine in rats [in Japanese]. Oyo Yakuri Pharmacometrics. 1978;15(2):199–221.

4.1.2 Phenol and phenol derivatives

Introduction

The Committee evaluated an additional seven flavouring agents in the group of phenol and phenol derivatives for the first time. In addition, the Committee considered new data for four previously evaluated flavouring agents in this group and data on oregano oil with the principal constituents carvacrol (No. 710) and thymol (No. 709).

The Committee evaluated 48 members of this group of flavouring agents at its 55th meeting (Annex 2, reference *149*), 13 members at its 73rd meeting (Annex 2, reference *202*), 3 members at its 76th meeting (Annex 2, reference *211*) and 4 members at its 79th meeting (Annex 2, reference *220*). The Committee concluded that none of the 68 flavouring agents was a safety concern at the estimated dietary exposures.

The additional flavouring agents in this group evaluated at the present meeting are (±)-homoeriodictyol sodium salt (No. 2256), (±)-naringenin (No. 2257),

(2*R*)-3′,5-dihydroxy-4′-methoxyflavanone (No. 2258), 7,8-dihydroxyflavone (No. 2259), (2*S*)-3′,7-dihydroxy-8-methyl-4′-methoxyflavan (No. 2260), (*R*)-5-hydroxy-4-(4′-hydroxy-3′-methoxyphenyl)-7-methylchroman-2-one (No. 2261) and 3-(3-hydroxy-4-methoxyphenyl)-1-(2,4,6-trihydroxyphenyl)propan-1-one (No. 2262). Four of these agents (Nos 2256, 2257, 2258 and 2260) have been reported to occur naturally in *Yerba santa*, citrus juices, *Sophora* (Fabaceae), *Dracaena cambodiana* and *Lycoris radiate (1–7)*.

Six of the seven additional members of this group were evaluated according to the Revised Procedure for the Safety Evaluation of Flavouring Agents (Annex 2, reference *230*).

Assessment of dietary exposure

The total annual volume of production of the seven additional flavouring agents in the group of phenol and phenol derivatives is 937 kg in the USA and 1050 kg in Latin America. More than 91.9% of the annual production volume in the USA is accounted for by 3-(3-hydroxy-4-methoxyphenyl)-1-(2,4,6-trihydroxyphenyl) propan-1-one (No. 4872), and 100% of the annual production volume in Latin America is accounted for by (±)-naringenin (No. 4797).

Dietary exposures were estimated by both the SPET and the MSDI method; the higher of the two values for each flavouring agent is reported in Table 3. The SPET and MSDI method values are in the range of 360–12000 and 0.01–89 μg/day, respectively. The estimated daily dietary exposure was highest for (±)-naringenin (No. 4797) (the SPET value obtained for non-alcoholic soft beverages).

Absorption, distribution, metabolism and elimination

Information on the ADME of flavouring agents in the group of phenol and phenol derivatives is provided in the monographs of the 55th, 73rd, 76th and 79th meetings (Annex 2, references *174*, *203*, *212* and *221*). Additional information was available for this meeting on three of the new flavouring agents (Nos 2257, 2260 and 2261).

Glycoside conjugates of polyphenols are hydrolysed on the brush border of or within small intestine epithelial cells. Polyphenols are rapidly but incompletely absorbed after oral administration and metabolized in the gastrointestinal tract and the liver. Polyphenols are metabolized by hydrolysis, sulfation, glucuronidation and/or methylation. Those conjugates excreted into urine are eliminated rapidly. The molecular weight of some conjugates is high enough that a portion is also excreted into bile. The biliary metabolites can undergo enterohepatic circulation (transfer into the intestine, hydrolysis, reabsorption and reconjugation primarily in the liver, with excretion in urine), which accounts

Table 3

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

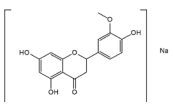
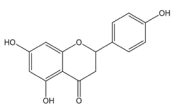
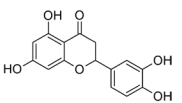
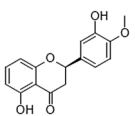
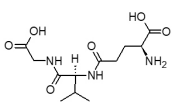
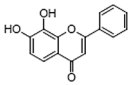
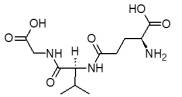
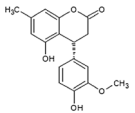
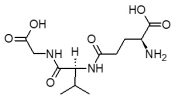
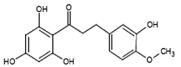
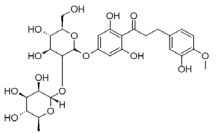
Flavouring agent	No.	CAS No. and structure	Step 4 Does intake exceed the threshold of toxicological concern?[e]	Step 5 Does a NOAEL exist for the flavouring agent or a structural relative that provides an adequate MOE?	Comments on predicted metabolism	Structural relative name (No.) and structure	Conclusion based on current estimated dietary exposure
Structural class II							
(±)-Homoeriodictyol, sodium salt	2256	462631-45-4 	No	NR	See note 1	NR	No safety concern
Structural class II							
(±)-Naringenin	2257	67604-48-2; 17654-19-2; 480-41-1 	Yes, SPET 12 000	Yes, The NOAEL of 968 mg/kg bw per day for structurally related (±)-eriodictyol (No. 2172) in a 90-day study in rats *(10)* is 4800 times the estimated dietary exposure of No. 2257 when used as a flavouring agent.	See note 1	(±)-Eriodictyol (No. 2172) 	No safety concern
(2*R*)-3´,5-Dihydroxy-4´-methoxy-flavanone	2258	1449417-52-0 	Yes, SPET 6000	Yes, The NOAEL of 968 mg/kg bw per day for structurally related (±)-eriodictyol (No. 2172) in a 90-day study in rats *(10)* is 9600 times the estimated dietary exposure of No. 2258 when used as a flavouring agent.	See note 1	Glutamyl-valyl-glycine (No. 2123) 	No safety concern

Table 3 (continued)

Flavouring agent	No.	CAS No. and structure	Step 4 Does intake exceed the threshold of toxicological concern?[e]	Step 5 Does a NOAEL exist for the flavouring agent or a structural relative that provides an adequate MOE?	Comments on predicted metabolism	Structural relative name (No.) and structure	Conclusion based on current estimated dietary exposure
7,8-Dihydroxy-flavone	2259	38183-03-8 	Yes, SPET 2000	Yes, The NOAEL of 968 mg/kg bw per day for structurally related (±)-eriodictyol (No. 2172) in a 90-day study in rats *(10)* is 29 500 times the estimated dietary exposure of No. 2259 when used as a flavouring agent.	See note 1	Glutamyl-valyl-glycine (No. 2123) 	No safety concern
(*R*)-5-Hydroxy-4-(4´-hydroxy-3´-methoxyphenyl)-7-methyl-chroman-2-one	2261	1793064-68-2 	Yes, SPET 4500	Yes, The NOAEL of 968 mg/kg bw per day for structurally related (±)-eriodictyol (No. 2172) in a 90-day study in rats *(10)* is 12 900 times the estimated dietary exposure of No. 2261 when used as a flavouring agent.	See note 1	Glutamyl-valyl-glycine (No. 2123) 	No safety concern
3-(3-Hydroxy-4-methoxyphenyl)-1-(2,4,6-trihydroxyphenyl) propan-1-one	2262	35400-60-3 	Yes, SPET 3000	Yes. The NOAEL of 750 mg/kg bw per day for structurally related neohesperidin dihydrochalcone in a 90-day study in rats *(11)* is 15 000 times the estimated dietary exposure of No. 2262 when used as a flavouring agent.	See note 1	Neohesperidin dihydrochalchone 	No safety concern

[a] In total, 68 flavouring agents were previously evaluated by the Committee, at its 55th, 73rd, 76th, and 79th meetings (Annex 2, references *149*, *202*, *211* and *220*).

[b] Step 1: The weight of evidence is that none of the chemical-specific genotoxicity data for the above five flavouring agents indicates that they have the potential to be DNA-reactive carcinogens.

[c] Step 2: One flavouring agent is in structural class II (No. 2256), and five are in structural class III (Nos. 2257, 2258, 2259, 2261 and 2262). Whereas naringin, the 7-O-glycoside between the flavanone naringenin and the disaccharide neohesperidose, is a common constituent of citrus fruit juices and is responsible for the bitter taste of grapefruit, the aglycone (±)-naringenin (No. 2257) is not a common constituent of foods. Hence, it is classified in structural class III.

[d] Step 3: Dietary exposures were estimated with both the SPET and the MSDI method, and the higher of the two values for each flavouring agent is reported in the table. The SPET gave the highest estimate for each flavouring agent. All dietary intake values are expressed in µg/day.

[e] The thresholds of toxicological concern for structural classes II and III are 540 and 90 µg/day, respectively.

Note:

1. Aglycones are demethylated or methylated and form sulfates or glucuronic acid conjugates before elimination in the urine or bile.

for the relatively slow urinary excretion observed in some cases. Metabolites that are not hydrolysed and absorbed in the small intestine may undergo further metabolism in the large intestine, where the microflora cleave conjugates and the resulting aglycones undergo ring cleavage, leading to phenolic acid and cinnamic acid derivatives, which are also ultimately excreted in the urine.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. There are no structural alerts for genotoxicity for the additional flavouring agents (Nos 2256, 2257, 2258, 2259, 2260, 2261 and 2262) in this group. Chemical-specific genotoxicity data on previously evaluated flavouring agents in this group and on six of the seven new flavouring agents (Nos 2256–2259, 2261 and 2262) do not indicate that they have genotoxic potential; however, the Committee was concerned about genotoxicity for one of the seven new flavouring agents (No. 2260), which was therefore not further considered with the Revised Procedure for the Safety Evaluation of Flavouring Agents.

Step 2. In applying the Revised Procedure to the six remaining flavouring agents (Nos 2256–2259, 2261 and 2262), the Committee assigned one agent (No. 2256) to structural class II and five (Nos 2257–2259, 2261 and 2262) to structural class III *(8)*.

Step 3. Dietary exposures have been determined with the MSDI method and SPET.

Step 4. The higher estimated dietary exposure for the one flavouring agent in structural class II (No. 2256) is below the threshold of toxicological concern (i.e. 540 µg/person per day). The Committee therefore concluded that flavouring agent No. 2256 would not pose a safety concern at the currently estimated level of dietary exposure.

The highest estimated dietary exposures of the five flavouring agents in structural class III are above the threshold of toxicological concern (i.e. 90 µg/person per day), and their evaluation proceeded to Step 5 of the Revised Procedure.

Step 5. For (±)-naringenin (No. 2257), the NOAEL of 968 mg/kg bw per day for the structurally related substance (±)-eriodictyol (No. 2172) in a 90-day dietary study in male and female rats *(9)* provides an adequate MOE (4800) relative to the SPET estimate of 12 000 µg/day. The NOAEL of 968 mg/kg bw per day for (±)-eriodictyol (No. 2172) is also appropriate for assessment of the structurally related flavouring agents (2*R*)-3′,5-dihydroxy-4′-methoxyflavanone (No. 2258), 7,8-dihydroxyflavone (No. 2259) and (*R*)-5-hydroxy-4-(4′-hydroxy-3′-methoxyphenyl)-7-methylchroman-2-one (No. 2261). The NOAEL of 986 mg/kg bw per day provides adequate MOEs of 9600, 29 500 and 12 900 relative

to the SPET estimates of 6000, 2000 and 4500 μg/day, respectively, for these substances when used as flavouring agents.

For 3-(3-hydroxy-4-methoxyphenyl)-1-(2,4,6-trihydroxyphenyl) propan-1-one (No. 2262), the NOAEL of 750 mg/kg bw per day for the structurally related substance neohesperidin dihydrochalcone in a 90-day dietary study in male and female rats *(10)* provides an adequate MOE (15 000) relative to the SPET estimate of 3000 μg/day when it is used as a flavouring agent.

Table 3 summarizes the evaluations of the six flavouring agents in the group of phenol and phenol derivatives (Nos. 2256–2259, 2261 and 2262).

Consideration of combined intakes from use as flavouring agents

The Committee previously considered the potential combined intake of this group of phenol and phenol derivatives and did not identify any safety concern. As the MSDI values for the six additional flavouring agents in this group (Nos. 2256–2259, 2261 and 2262) are low (0.01–89 μg/day), they would make a negligible contribution to combined intake of this group.

Consideration of secondary components

One flavouring agent in this group (No. 2256) has a minimum assay value of < 95% (see Annex 4). The major secondary components (±)-eriodyctiol-7-methyl ether, present at 3–5%, and (±)-homoeriodictyol-7-methyl ether, present at 1–2%, are structurally related to (±)-eriodictyol (No. 2172). These secondary compounds are considered not to present a safety concern when consumed as components of No. 2256 used as a flavouring agent at its current estimated dietary exposure.

Consideration of additional data on previously evaluated flavouring agents

The Committee considered additional data on four previously evaluated flavouring agents in this group. New studies of short-term toxicity (No. 2172 and oregano oil with the primary constituents Nos 709 and 710) and of genotoxicity (Nos 702, 709 and 710) support the conclusions of the previous evaluations that these flavouring agents are not safety concerns.

Conclusions

In the previous evaluations of 68 substances in this group of phenol and phenol derivatives, studies of ADME, acute toxicity, short-term and long-term toxicity and genotoxicity were available (Annex 2, references *174*, *203*, *212*, *221*). None of the agents in this group raised a safety concern.

For the evaluation of six further flavouring agents, studies of ADME (Nos. 2257 and 2261) and of genotoxicity (Nos 2259, 2261 and 2262) were

available. Studies of ADME and genotoxicity were also available for No. 2260, for which the evaluation was not completed at this meeting.

The Committee concluded that the six flavouring agents (Nos 2256, 2257, 2258, 2259, 2261 and 2262) would not give rise to safety concerns at the current estimated dietary exposures.

The Committee also concluded that the additional data presented in this addendum further support the safety of the 68 previously evaluated flavours in this group.

For (2S)-3′,7-dihydroxy-8-methyl-4′-methoxyflavan (No. 2260), clear positive findings were observed in an *in vitro* micronucleus test that were not resolved by the results of an *in vivo* bone marrow micronucleus test in mice, in which no proof of systemic or target tissue exposure was provided. The evaluation of flavouring agent No. 2260 was not completed because of the genotoxic concern, and additional investigation is required.

An addendum to the monograph was prepared.

References

1. Johnson ND. Flavanoid aglycones from Eriodyctyon californicum resin and their implications for herbivory and UV screening. Biochem Syst Ecol. 1983;11(3):211–5.
2. Numata A, Takemura T, Ohbayashi H, Katsuno T, Yamamoto K, Sato K, et al. Antifeedants for the larvae of the yellow butterfly, *Eurema hecabe mandarina*, in *Lycoris radiate*. Chem Pharm Bull. 1983;31(6):2146–9.
3. Ruiz E, Donoso C, Gonzales F, Becerra J, Marticorena C, Silva M. Phenetic relationships between Juan Fernandez and continental Chilean species of *Sophora* (Fabaceae) based on flavonoid patterns Bol Soc Chilena Quim. 1999;44:351–6.
4. Liu J, Dai HF, Wu J, Zeng YB, Mei WL, Flavanes from *Dracaena cambodiana*. Z Naturforsch C. 2008;63:1407–10.
5. Yáñez JA, Remsberg CM, Miranda ND, Vega-Villa KR, Andrews PK, Davies NM. Pharmacokinetics of selected chiral flavonoids: hesperetin, naringenin and eriodictyol in rats and their content in fruit juices. Biopharm Drug Dispos. 2008;29(2):63–82.
6. Liu J, Mei WL, Wu J, Zhao YX, Peng M, Dai HF. A new cytotoxic homoisoflavonoid from *Dracaena cambodiana*. J Asian Nat Prod Res. 2009;11(2):192–5.
7. Luo Y, Wang H, Xu X, Mei W, Dai H (2010) Antioxidant Phenolic Compounds of Dracaena Cambodiana. Molecules. 2010;15:8904-14.
8. Cramer GM, Ford RA, Hall RL. Estimation of toxic hazard – a decision tree approach. Food Cosmet Toxicol. 1978;16(3):255–76.
9. Bauter M. Eriodictyol Nat. 98%: A 90-day dietary study in rats. Study number 45619. Product Safety Labs, Dayton, NJ. Unpublished report to the Expert Panel of the Flavor and Extract Manufacturers Association. Submitted to WHO by the International Organization of the Flavor Industry, Brussels, Belgium; 2018.

10. Lina BAR, Dreef-van der Meulen HC, Leegwater DC. Subchronic (13-week) oral toxicity of neohesperidin dihydrochalcone in rats. Food Chem Toxicol. 1990;2:507–13.

4.2 Specifications of identity and purity

4.2.1 New specifications

The Committee received information related to specifications for all 15 of the new flavouring agents for which there had been a call for data for the present meeting. Full specifications were prepared for 12 of the agents. Tentative specifications were prepared for three: No. 2260 in phenol and phenol derivatives and Nos 2263 and 2264 in alicyclic ketones, secondary alcohols and related esters, as the safety evaluations for these flavouring agents were not completed, which is noted in all three tentative specifications.

The Committee considered the best approach for assigning molecular weight to flavourings. It was decided that, for future evaluations, the molecular weight reported by CAS SciFinder, when available, would be used as the default molecular weight.

4.2.2 Revised specifications

The Committee received information for revision of the full specifications for 14 flavouring agents that were on the agenda of the present meeting (JECFA Nos 2002, 1575, 1604, 2077, 1125, 380.1, 1491, 1497, 1502, 1504, 1506, 1511, 1513 and 1517).

The specifications for 4-hydroxy-2,3-dimethyl-2,4-nonadienoic acid γ-lactone (No. 2002) were revised with data on eight lots of commercial product. The specific gravity was revised to 0.950–1.000 at 20 °C, and the assay minimum was maintained at 93%, with a change of the secondary component from 1–2% 3,4-dimethyl 5-ketobutanoic acid γ-lactone to 2–3% 3,4-dimethylfuran-2,5-dione. The new secondary component was not considered a safety concern, as noted in Annex 4.

For β-caryophyllene oxide (No. 1575), the Committee revised the melting-point to 55–63 °C and the assay minimum to 95% (sum of isomers) from data on eight lots of commercial product. Specifications for the isomeric compositions were also established: 84–89% 1*R*,4*R*,6*R*,10*S* (CAS No. 1139-30-6), 7–9% 1*R*,4*R*,6*S*,10*S* (CAS No. 60594-22-1), 0.3–2% 1*R*,4*S*,6*S*,10*S* (CAS No. 103475-43-0) and 1–2% humulene-1,2-epoxide (CAS No. 19888-34-7).

For 2-acetyl-1-pyrroline (No. 1604), the Committee revised the assay minimum to 90% from data on three lots of commercial product, with a secondary component of up to 5–6% of 5,6-dihydro-2-methyl-3-(4*H*)-pyridinone. The secondary component was not considered a safety concern, as noted in Annex 4.

The Committee updated the isomeric composition of (2*E*,6*E*/*Z*,8*E*)-*N*-(2-methylpropyl)-2,6,8-decatrienamide (No. 2077) from data on 25 lots of commercial product as follows: 73–80% 2*E*,6*Z*,8*E*, 15–18% 2*E*,6*E*,8*E*, 3–7% 2*E*,6*Z*,8*Z*, 1–2% 2*Z*,6*Z*,8*E* and 1–2% 2Z,6*E*,8*E*.

For 4-hexen-3-one (No. 1125), the Committee revised the assay minimum to 95% (sum of isomers) from data on seven lots of commercial product. Specifications for the isomeric composition were also established: 90–95% *trans*-4-hexen-3-one and 1–5% *cis*-4-hexene-3-one.

For d-carvone (No. 380.1), the Committee revised the refractive index to 1.496–1.502 and the specific gravity to 0.956–0.961 from data on 30 lots of commercial product. The specifications were maintained as tentative.

For 2-pentylfuran (No. 1491), the Committee revised the refractive index to 1.445–1.451 and the assay minimum to 95% from data on 30 lots of commercial product.

For 3-(2-furyl)acrolein (No. 1497), the Committee revised the melting point to 42–54 °C from data on 25 lots of commercial product.

The Committee revised the specifications for 2-phenyl-3-(2-furyl)prop-2-enal (No. 1502) from data on 15 lots of commercial product and revised the physical form and odour.

For 2-acetyl-5-methylfuran (No. 1504), the Committee reviewed data on 91 lots of commercial product and revised the specific gravity to 1.065–1.074 and the assay minimum to 95% and revised the physical form and odour.

For 3-acetyl-2,5-dimethylfuran (No. 1506), the Committee reviewed data on 40 lots of commercial product and revised the specific gravity to 1.034–1.048 and revised the physical form and odour.

For 4-(2-furyl)-3-buten-2-one (No. 1511), the Committee reviewed data on 37 lots of commercial product and revised the melting-point to 28–40 °C and revised the physical form and odour.

The Committee revised specifications for ethyl 3-(2-furyl) propanoate (No. 1513) from data on 48 lots of commercial product. The physical form and odour were revised, and specifications for the refractive index and the specific gravity were established as 1.455–1.462 and 1.051–1.058, respectively.

The Committee revised specifications for phenethyl 2-furoate (No. 1517) from data on 13 lots of commercial product. The refractive index was revised to 1.540–1.550 and the specific gravity to 1.138–1.150, and the physical form and odour were updated.

5. Future work and recommendations

5.1 Carbohydrate-derived fulvic acid

The Committee requires data to characterize the products of commerce in order to evaluate the product for use as a preservative. The required information includes a detailed description of the manufacturing processes and thorough chemical characterization of the commercial products.

The following information is required:

- the full composition of the products;
- a detailed description of the manufacturing process;
- analytical methods and data on method validation; and
- analytical data for five non-consecutive batches of commercial products, including information on impurities.

The sponsor is encouraged to offer a rationale for whether a single monograph covering all products or individual monographs should be prepared.

Given the deficiencies of the toxicological database, the Committee recommends that the following studies be conducted. The test protocols should be in accordance with the relevant current guidelines, and the test materials should be well characterized in relation to the article(s) of commerce:

- absorption, distribution, metabolism and excretion;
- repeated-dose 90-day oral toxicity in rodents;
- two-generation reproductive toxicity or extended one-generation reproductive toxicity;
- prenatal developmental toxicity;
- additional studies, including an in vitro micronucleus test in mammalian cells, might be required, depending on elucidation of the article(s) of commerce and the provision of full information on their composition; and
- information on the potential of the material to induce antimicrobial resistance.

In addition, use levels should be provided for estimating dietary exposure.

5.2 Withdrawal of the ADI for lipase from Aspergillus oryzae, var.

The Committee also noted that specifications for other food additives had been withdrawn at the 55th meeting without addressing the consequences for the respective ADIs. The Committee recommends reconsideration of the ADIs concerned at a future meeting.

5.3 Riboflavin from *A. gossypii*

The Committee drafted a chemical and technical assessment and new specifications for riboflavin from *A. gossypii* from the data submitted by the sponsor, but did not finalize them for publication. The Committee recognized the benefits of simultaneous review and harmonization of new specifications with existing specifications for riboflavin as a synthetic product and as a product of *B. subtilis* and recommended that this work be undertaken at a future meeting.

5.4 Sucrose esters of fatty acids (INS 473) and sucrose oligoesters types I and II (INS 473a)

To refine the dietary exposure estimates of SEFs and SOEs, either alone or summed, the Committee recommends that sponsors submit information on:

- typical or mean and high use levels for foods in which the food additives are used; and
- foods (or food categories) in which the use of SEFs and/or SOEs is permitted but in which they are never used.

In both cases, the information should be as specific as possible, and the foods should be classified according to the FoodEx2 classification system, which is that used for the CIFOCOss and GIFT food consumption databases, or another appropriate system.

The Committee did not use the CIFOCOss and GIFT databases to assess dietary exposure to SEFs and SOEs, partly because calculations of exposure would have been laborious in view of the number of broad food categories for which use levels were provided. In order to use these data for dietary exposure assessment of food additives that are present in large numbers of food categories, a table should be developed to map the foods recorded in both databases according to the FoodEx2 classification to the food categories of the GSFA. That will also ensure that mapping is consistent for all meetings.

The Committee recommends that more detailed information on the use of SEFs and SOEs in foods and a mapping table be made available within 2 years.

5.5 Polyvinyl alcohol

The Committee recommended that the CCFA determine whether the food-grade PVOH products currently available in commerce comply with the narrow range of viscosity (4.8–5.8 mPa × s) and degree of hydrolysis (86.5–89%) in the specifications. Any deviations would necessitate a review of its safety evaluation.

The Committee also noted that the gas chromatographic method for determining methanol and methyl acetate in PVOH is a packed-column method and recommended that it be replaced by a suitable capillary or wide-bore column gas chromatographic method.

5.6 Sorbitan esters of fatty acids (INS 491, INS 492 and INS 495)

The Committee recommends that a new call for data be issued in order to proceed with an updated safety evaluation and specifications for the five sorbitan esters of fatty acids at the same time.

The Committee also noted that five polyoxyethylene sorbitan esters (polysorbates) were evaluated by JECFA at its 17th meeting Annex 1, reference *32*), and specifications were established. The Committee recommends that a new call for data be issued for their full evaluation.

Acknowledgements

FAO and WHO acknowledge the significant contributions of the experts, as well as their institutions (where relevant), to the work of the eighty-ninth meeting of JECFA. The Committee thanks Ms Elisabeth Heseltine, Saint Léon-sur-Vézère, France, for her assistance in the preparation of the report.

Corrigenda

The following requests for corrections, reported to the JECFA Secretariat, were evaluated by JECFA at the current meeting and found to be necessary. The corrections will be made, however, only in the electronic versions and in the online database.

Food additive	Original text	Revised text	Additional information
Calcium disodium ethylenediamine-tetraacetate INS 385	CAS No. 662-33-9	CAS No. 62-33-9 (anhydrous) 6766-87-6 (dihydrate) 23411-34-9 (hydrated)	Correction to CAS No. (for the anhydrous form)
	Chemical formula C10H12CaN2Na2O8 . 2H2O	Chemical formula C10H12CaN2Na2O8 (anhydrous) C10H12CaN2Na2O8 · H2O (monohydrate) C10H12CaN2Na2O8 · 2H2O (dihydrate)	CAS No. for hydrated forms; chemical formula and formula weight for anhydrous and monohydrate also included
	Formula weight 410.31	Formula weight 374.37 (anhydrous) 392.31 (monohydrate) 410.31 (dihydrate)	
Pentasodium triphosphate INS 451(i)	Dowex F x 8	Dowex 1 x 8	Correction to the resin in the procedure of method of assay
Talc INS 553(iii)	A range of length:width ratios of 20:1 to 100:1 or higher for fibres longer than 5 m	A range of length:width ratios of 20:1 to 100:1 or higher for fibres longer than 5 µm	Length of fibre corrected
Annatto extracts (norbixin-based) INS 160b(ii) 1. Alkali processed norbixin, acid precipitated 2. Alkali processed norbixin, not acid precipitated 3. Solvent extracted norbixin	CAS Nos *cis*-Norbixin: 542-40-5 *cis*-Norbixin dipotassium salt: 33261-80-2 *cis*-Norbixin disodium salt: 33261-81-3	CAS Nos *cis*-Norbixin: 626-76-6 *cis*-Norbixin dipotassium salt *cis*-Norbixin disodium salt:	Correction to the CAS No. of *cis*-norbixin and deletion of the incorrect CAS Nos for the dipotassium and disodium salts

Annex 1

Revision of Environmental Health Criteria 240 for the evaluation of enzyme preparations used in the manufacture of foods

Issued in September 2020 by the Food and Agriculture Organization of the United Nations and the World Health Organization

1. Revision of Chapter 9.1.4.2. Enzymes

The history of enzyme use in food applications is long and well known, especially in bread-, cheese-, wine- and beer-making, where enzymes are part of the processing or maturation processes. Enzymes used in the food industry are produced from animal tissues, plants and microorganisms; however, most commercial enzymes are produced from microorganisms that have been enhanced through natural selection, classical strain improvement techniques (e.g. mutagenesis and selection), recombinant-DNA technologies and gene editing. Microbial enzymes are typically produced by controlled fermentation, followed by removal of the production microorganism, purification and concentration of the enzyme. The enzyme is finally standardized with stabilizers, preservatives, carriers, diluents and other approved food-grade additives and ingredients. The formulated enzymes are referred to as "enzyme preparations", which, depending on the application, may be produced as a liquid, semi-liquid or dried product. Enzyme preparations may contain either one major active enzyme that catalyses a specific reaction or two or more active enzymes that catalyse different reactions during food processing.

Enzyme preparations often contain organic constituents of the production organism and compounds carried over from the manufacturing process, such as residues of the fermentation broth. In 2006, the sixty-fifth JECFA Committee elaborated principles and procedures for the safety assessment of enzyme preparations for use in food, whereby, an enzyme preparation must comply with the *General Specifications and Considerations for Enzyme Preparations Used in Food Processing (1)*. The document addresses certain aspects that apply to the safety evaluation of all enzyme preparations, including of the production organism, the enzyme component(s), side activities, the manufacturing process and consideration of dietary exposure.

Specific safety concerns are possible allergic reactions to the enzyme and about enzyme preparations produced by genetically modified microorganisms.

1.1 Potential allergic reactions

1.1.1 Food allergies

Food allergies are adverse immunological reactions to an otherwise harmless food, such as a protein. The severity of food allergies in susceptible individuals (atopy) ranges from mild to severe and in some cases may be life-threatening. The most common type of food allergy is mediated by allergen specific immunoglobulin E antibodies. Most allergens are proteins (e.g. Ara h2 in peanuts, papain in papaya, lacto-peroxidase in cow's milk), but not all food proteins are allergens. As no single test can accurately predict whether a microbially synthesized enzyme will immunologically cross-react with an established allergen, a weight-of-evidence approach should be used *(2)*. One that is routinely used by JECFA is comparison of the amino acid sequence of an enzyme against known linear immunoglobulin E-binding epitopes in allergenic proteins *in silico* and with appropriate protein databases, such as Food Allergy Research and Resource Program, University of Nebraska, and AllergenOnline (http://www.allergenonline.org). The possibility of immunological cross-reactivity between the expressed enzyme and a known allergen is considered when there is:

- at least 35% identity of the amino acid sequence of the expressed protein (i.e. without the leader sequence, if any) with a sliding window of 80 amino acids and a suitable gap penalty (for algorithms such as FASTA, BLASTP *(3)* or equivalent); and
- identification of eight contiguous amino acids common to the expressed enzyme and a known allergen *(4)*.

Information on the amino acid sequences is not available for most enzymes derived from animals or plants or produced by microorganisms that are accepted constituents of foods. Thus, absence of allergenicity in humans is considered to have been demonstrated by their presence in widely consumed foods for a long time.

1.1.2 Allergenic food proteins and resistance to proteolysis

The susceptibility of a dietary protein to proteolytic degradation by digestive enzymes such as gastric pepsin could provide information on its immunological safety for human consumption. While most dietary proteins are readily hydrolysed to peptides and amino acids in the gastrointestinal tract, there is evidence that many potent food allergens are resistant to proteolysis *(2, 5–7)*. *In vitro* pepsinolysis assays *(8)* have been proposed to provide additional information for a weight-of-evidence approach for newly expressed proteins *(9)*. A pepsinolysis assay based on simulated gastric fluid that is frequently used in pre-clinical testing of pharmaceuticals has been described by the United States Pharmacopeia *(10)* and

is often used to compare different newly expressed proteins under experimental conditions i. To date, however, such data on pepsin resistance for enzymes have rarely been submitted to JECFA for consideration in a weight-of-evidence approach, perhaps because studies conducted under different conditions of pH, purity and activity of pepsin and pepsin-to-substrate protein ratio have shown no absolute correlation with allergenic potential and that proteins that are resistant to pepsinolysis might not be allergenic under physiological conditions of dietary exposure, whereas labile proteins (e.g. β-casein) or peptides formed during proteolysis may be allergenic *(12–16)*. Consequently, data from *in vitro* tests on resistance to pepsinolysis are currently not considered to be strong evidence for the absence of intrinsic allergenicity of a protein, although they may have some utility as part of a weight-of-evidence approach.

1.1.3 Occupational hazards: respiratory allergies, skin and eye irritation

A known safety risk linked to industrial use of enzymes is respiratory allergy, and most proteases also have some potential for skin and eye irritation *(17, 18)*. Enzymes present a risk of a respiratory allergy (e.g. *Aspergillus*-derived enzymes in bakers' asthma), which is well described in the scientific literature *(19, 20)*.

1.2 Safety concerns about enzyme preparations produced by genetically modified microorganisms

The *General Specifications and Considerations for Enzyme Preparations Used in Food Processing (1)* include recommendations on the safety assessment of genetic material inserted into the genome of a production microorganism. Two additional considerations that were introduced in the 2006 revision of the document are:

> For enzyme preparations from recombinant-DNA-modified microorganisms the genetic material introduced into and remaining in the production microorganism should be characterized and evaluated for function and safety, including evidence that it does not contain genes encoding known virulence factors, protein toxins, and enzymes involved in the synthesis of mycotoxins or other toxic or undesirable substances.
>
> Recombinant-DNA-modified production micro-organisms might contain genes encoding proteins that inactivate clinically useful antibiotics. Enzyme preparations produced with such microorganisms should contain neither antibiotic inactivating proteins at concentrations that would interfere with antibiotic treatment nor transformable DNA

that could potentially contribute to the spread of antibiotic resistance.

Extensive literature on the safety of enzymes from microbial sources support the general assumption that industrial enzyme preparations from non-pathogenic organisms are safe *(21)*. The amino acid sequences of most engineered enzymes exhibit no greater variation than that of many isozymes in the diet *(22)*. Furthermore, there is no evidence that changes in amino acid sequence made through protein engineering, to confer benefits such as tolerance to heat and/or pH or simply to increase yield, will render an otherwise safe enzyme toxic. That said, comparison of the amino acid sequence of an enzyme with those of known toxic or allergenic proteins *in silico* can exclude the possibility that the enzyme is toxic, allergenic or has some other physiological effect.

1.3 Toxicological assessment of enzyme preparations

Enzyme preparations contain either one major active enzyme that catalyses a specific reaction or two or more active enzymes that catalyse different reactions during food processing. Each enzyme in the preparation must comply with the established specifications for identity and purity.

While food enzyme preparations are considered unlikely to cause any acute toxicity, genotoxicity or toxicity after repeated oral doses, the fermentation product(s) of microorganisms from the manufacturing process may be of interest because of the potential presence of secondary metabolites that may induce toxicity when ingested (e.g. aflatoxins, fumonisins and/or ochratoxins) *(23)*. The fermentation product, which also includes the food enzyme of interest, has usually been tested for genotoxicity and in repeat-dose rodent feeding studies submitted to JECFA.

The *General Specifications and Considerations for Enzyme Preparations Used in Food Processing* published by FAO *(1)* and the Scientific Committee on Food *(24)* lists the points of potential toxicological concern, noting the following.

- Different strains belonging to the same species may behave differently. For many microorganisms, it is known that some strains of a species are harmless, while others ofthe same species may produce toxins.
- Isolates of some fungal genera, especially *Penicillium* and *Aspergillus*, have often been misidentified. Consequently, fungal strains may be misclassified. For example, it has sometimes been difficult to distinguish *A. oryzae* from *A. flavus*; the latter may produce aflatoxins. As there is a risk that microbial isolates may be misidentified, the microorganism used must be correctly identified, and, in case of

doubt, the identity should be verified by an recognized, independent laboratory.

- The ability of microorganisms to turn on genes that code for toxins may depend on fermentation conditions, such as the composition of the media, pH, temperature and fermentation period. Therefore, a microorganism that does not produce toxins under some conditions may produce toxins under others.
- The continuous selection processes applied to source microorganisms in order to maximize and optimize enzyme production may result in spontaneous mutations that could change a non-toxin-producing strain into a toxin-producing strain, if its genetic predisposition is such that these mutations are sufficient to turn on the expression of pre-existing toxin-producing genes.
- New techniques of genetic modification are available for use in the production of food enzymes. With the introduction of desirable traits, genes can be introduced or deleted for toxin production. Therefore, the genetic construct in the host, vector and insert must be explicitly characterized and evaluated.

As a result of these safety concerns, the toxicological testing requirements are:

- For enzymes produced from edible parts of animals or plants, no toxicological tests are normally required. When enzymes are obtained from parts that are not generally considered part of the normal diet, however, some toxicological testing may be required, unless other satisfactory documentation of safe use is provided.
- For enzyme preparations produced by microorganisms, toxicological tests shall, where possible, be performed on batches of the final purified, concentrated fermentation product before the addition of formulation ingredients (e.g. carriers, diluents). The following tests are usually required:
    - 90-day oral toxicity test in a rodent species; and
    - two short-term tests: for gene mutations in bacteria and for chromosomal aberrations (preferably in vitro).

1.3.1 Dietary exposure and margin of exposure

Dietary exposure is calculated on the basis of the total organic solids (TOS) content in the final (commercial) enzyme preparation and is usually expressed in milligrams or micrograms TOS per kilogram body weight per day. TOS

encompasses the enzyme component and other organic material originating from the production organism and the manufacturing process, while excluding intentionally added formulation ingredients. JECFA then considers the estimated dietary exposure to an enzyme preparation according to the proposed uses and levels of use in food and relates it to the NOAEL in its hazard assessment in order to determine a margin of exposure (MoE).

1.3.2 Exemptions from the basic toxicological requirements

The original guidelines listed exemptions from performing toxicological bioassays in the safety assessments of enzymes.

- "From a toxicological point of view, it is important to perform a toxicological testing procedure on each specific enzyme preparation produced from a microbiological source. If, however, one enzyme from a specific strain has been thoroughly tested and the manufacturing process does not differ significantly for other enzymes from the same strain, the full testing battery may be waived for such enzymes. This will be decided on a case-by-case basis" *(24)*.

- "If the microorganism used in the production has a long history of safety in food use, belongs to a species that has been well-documented, does not produce toxins, and the strain itself is of well documented origin, the acceptance of an enzyme preparation from such a microorganism with no specific toxicological testing may be justified. In this case, a correct and confirmed identification of the microorganism is of paramount importance" *(24)*.

Thus far, very few examples of these exemptions from toxicological testing have been considered in safety assessments of enzymes by JECFA. This may be because of uncertainty about compliance with the requirements to accurately identify the microbial strain and to assess the ability of the microorganism to produce toxins. These requirements can be met more easily with current technologies such as analytical molecular biology techniques, such as full genome sequencing, gene probing or RNA-sequencing technologies, to minimize misidentification *(25)* and chemometrics *(26)* to identify and quantify secondary metabolites in complex mixtures of natural products that may result from microbial fermentation.

If sponsors do not conduct toxicity testing, they are obliged to provide other information to attest to the enzyme's safety. The full battery of toxicological testing may be waived for enzymes from a specific (new) strain if the manufacturing process does not differ significantly for other enzymes of the same strain, a related strain or a lineage of related strains, provided other

evidence is presented to support the safety of the enzyme preparation of interest (for example, chemical assessment for known toxins, whole-genome sequencing and assessment for possible toxin production).

1.4 Classification of enzymes

To aid in decision-making, JECFA in 2018 reassessed the requirements for toxicological testing of enzyme preparations used in food and updated the classes as follows.

Class I: Enzymes obtained from sources that are considered safe for consumption and for which toxicological evaluations are NOT normally required

This class, which also includes immobilized enzymes from these sources, can be further categorized into:

Type i: Enzymes obtained from edible tissues of plants or animals commonly used as foods, which are regarded as foods and, consequently, their safety is considered acceptable, provided that satisfactory chemical and microbiological specifications can be established (e.g. papain, rennet). Use and use levels should be considered.

Type ii: Enzymes produced by microorganisms that are traditionally accepted as constituents of foods or are normally used in the preparation of foods (for example, *Saccharomyces* spp.).

These products are regarded as foods and, consequently, their safety is considered acceptable, provided that satisfactory chemical and microbiological specifications can be established. Enzymes produced by microorganisms modified by genetic engineering are not considered to be Class I type ii but fall into either Class I type iii or Class II. Use and use levels should be considered.

Type iii: Enzymes produced by a *safe food enzyme production strain* or a *presumed safe progeny strain* (for definitions, see below). For food enzyme preparations in this group, a detailed chemical and microbiological (genomic) analysis that confirms that the enzyme is produced by an organism that meets the definition of a safe food enzyme production strain or a presumed safe progeny strain that has undergone appropriate toxicological testing (i.e. repeat dose toxicity and genotoxicity testing) is required. Appropriate toxicological testing includes studies conducted on enzymes of other closely related strains derived from the same parental

organism. Published or unpublished genomic sequence data of the genetically modified microorganism could be provided to exclude the possibility of secondary metabolite toxin genes. Safety assessments of these food enzymes should also include appropriate information or other experimental data on their potential to cause an allergic reaction when ingested.

On completion of appropriate toxicological testing of the fermentation product from a production microorganism, the guidelines anticipate that it should be possible to conclude that the microorganism can be classified as a source considered safe for human consumption. Such a declaration was made for *A. oryzae* at the 68th meeting of JECFA in 2008 *(27)*. As of 2018, JECFA has evaluated over 80 food enzyme preparations from a variety of microorganisms and has never recorded a positive result in any toxicity study, suggesting that toxins were either not present or were present at levels that were below the limit of detection of the bioassays. These data suggest that many of the strains of microorganisms reviewed previously by JECFA (e.g. *Bacillus subtilis, B. licheniformis, Aspergillus niger* and *A. oryzae)* that are considered to be sources of food enzymes are safe for human consumption. Therefore, provided the genetic modification of the production organism, either as the result of the use of recombinant-DNA or chemical mutagenesis, is well characterized, additional toxicological testing is not required. Nevertheless, as described in Guidelines *(1)*, information on other aspects of enzyme production are still required. An ADI may be established.

Class II: Enzymes derived from sources that are NOT considered or presumed safe for consumption

Chemical and microbiological specifications must be established for all enzymes that do not fall under any of the sub-categories listed above. Similarly, relevant microbiological, toxicological and chemical data must be submitted for enzymes from organisms that have not been previously reviewed by JECFA, although they may subsequently be considered type 1(iii). Each enzyme will be evaluated, and an ADI may be established.

For enzymes produced by strains of microorganisms not previously evaluated by JECFA, information is required about the taxonomy, genetic background and other aspects related to the safety of the strain and commercial use in foods (if

any). Enzyme preparations produced by such microorganisms should contain neither antibiotic-inactivating proteins at concentrations that would interfere with antibiotic treatment nor transformable DNA that could potentially contribute to the spread of antibiotic resistance.

The absence of microorganism-derived secondary metabolites of toxicological significance in the enzyme concentrate should also be confirmed by submission of the results of two genotoxicity (mutagenicity and clastogenicity) assays on these enzymes, as well as a subchronic oral toxicity study. As an alternative to genotoxicity testing for the presence of secondary metabolites in the fermentation products, detailed chemical characterization of the enzyme concentrate, including confirmation of the absence of toxicologically significant levels of toxic secondary metabolites (e.g. mycotoxins that are known to be generated by strains of the production microorganism or by species related to the production microorganism), can be performed with high-performance liquid chromatography and/or mass spectrometry. Such characterization must be supported with a detailed genomic sequence of the genetically modified microorganism to exclude the possibility of the presence of genes capable of producing toxic secondary metabolites. Additional characterization of the enzyme protein would also be required, such as bioinformatics analyses to confirm the absence any potential allergenic epitopes or significant amino acid sequence homology to known toxins.

2. Definitions of "safe food enzyme production strain" and "presumed safe progeny strain" (Class I type iii)

A "safe food enzyme production strain" is a non-pathogenic, non-toxigenic microbial strain with a demonstrated history of safe use in the production of food enzymes. Evidence that would support a history of safe use includes taxonomy, genetic background, toxicological testing, other aspects related to the safety of the strain and commercial food use.

A "presumed safe progeny strain" is developed from a safe food enzyme production strain or from the parent of that strain. The progeny strain is developed by specific well-characterized modification of its genome; the modifications must be thoroughly documented, must not include encoding of

any harmful substance and not result in adverse effects. This concept also applies to multiple generations of progeny. Evidence supporting their safety includes taxonomy, genetic background and toxicological testing (including read-across of toxicological studies).

3. Information required for the safety assessment of enzyme preparations for use in foods

Class I: Enzymes obtained from sources that are considered safe for consumption and for which toxicological evaluations are NOT normally required

- Type i: Enzymes obtained from edible tissues of plants or animals commonly used as foods
- Type ii: Enzymes produced by microorganisms that are traditionally accepted as constituents of foods or are normally used in the preparation of foods
- Type iii: Enzymes produced by a safe food enzyme production strain or a presumed safe progeny strain I(iii).

Class II: Enzymes derived from sources that are NOT considered safe for consumption and are not in any of the sub-categories listed above

No.	Class	Information required	Details and rationale
Enzyme classification and description of active components of enzyme preparation			
1.	All	Name of enzyme(s)	e.g. triacylglycerol lipase
2.	All	Systematic name(s) and number(s)	EC/IUBMB Number; CAS Number (where appropriate)
3.	All	Molecular weight(s)	As determined by SDS PAGE, gel filtration chromatography etc.
4.	All	Amino acid sequence(s)	Predicted and determined primary amino acid sequence
5.	All	Catalytic activity	All reactions catalysed, including any secondary activities, conditions under which catalysis occurs, e.g. pH, temperature
6.	All	Known use(s) in food-based applications	Evidence of commercial food use, including from the parent strain or other strains in the lineage e.g. as a processing aid in the manufacture of bakery products, pasta and noodles, in egg yolk and in oil degumming
7.	All	Use levels in food(s)	Express each use as total organic solids (TOS) in mg/kg food, substrate or raw material; specify
8.	All	Fate in final food(s)	Is the enzyme active, inactive or removed? How is the enzyme inactivated or removed?
9.	All	Existing safety evaluations	Include any existing health-based guidance values (e.g. ADI)

No.	Class	Information required	Details and rationale
Details of the production organism			
10.	All	Identity of the production organism	Identify genus, species, strain
11.	I (iii), II	Host and recipient organism	Identify genus, species
12.	I (iii), II	Donor of genetic material	e.g., identify origins of genetic material by genus, species (if native or modified)
13.	I (iii), II	Details of genetic modification: To host genome	History of development of host strain (e.g. deletion of gene clusters that encode for aflatoxins, modifications that make host extracellular protease deficient or make it non-sporulating), identification of genes removed or added Donor of genetic material, details of how the genetic element was designed and the identity of genes on the element, stability information, copy numbers, whether it integrates or does not integrate into the host genome, etc.
		Addition of rDNA (gene of interest from another microorganism) to host microorganism through mobile genetic elements	Evidence that genetic material does not contain genes coding for virulence factors, protein toxins, or any enzymes that may be involved in the synthesis of mycotoxins
14.	I (iii), II	Genetic modification techniques	Site-directed mutagenesis, chemical mutagenesis, recombinant DNA technology, etc.
15.	I (iii), II	Description of intended and non-specific effects resulting from genetic modification and any changes made to prevent unwanted side reactions or products	e.g. an intended effect may be increased yield; a non-specific effect may be activation of toxin production. Rectification measures may include genetic modifications, specific fermentation conditions etc.
16.	All	Deposit information (if applicable)	e.g. ATCC number
Production of enzyme concentrate and preparation			
17.	All	Detailed manufacturing process	For enzymes in Class I(i) and Class I(ii) and Class II enzymes obtained from plants and animals, manufacturing details are required. For enzymes in Class I(iii) and Class II produced by microorganisms, include details of controlled fermentation inputs and conditions, steps taken to retain genetic modifications and further processing, purification and concentration steps. Indicate how production strains are maintained under conditions to ensure the absence of genetic drift, and, when used in the production of enzyme preparations, indicate the methods and conditions that are applied to ensure consistency and reproducibility from batch to batch. Such conditions must ensure the absence of toxin production by the organism and prevent the introduction of microorganisms that could be the source of toxic or other undesirable substances.
18.	All	Formulation ingredients	Identify the carriers, diluents, excipients, supports and other additives and ingredients (including processing aids) used in the production, stabilization and application of enzyme preparations, which must be acceptable for food use. In order to distinguish the proportion of the enzyme preparation arising from the source material from that contributed by diluents and other additives and ingredients, individual specifications require

No.	Class	Information required	Details and rationale
			a statement of percentage total organic solids (TOS), defined as follows: % TOS = 100 - (A + W + D) where A = % ash, W = % water, and D = % diluents and/or other additives and ingredients.
Specifications and data required for enzyme concentrates and preparations			
19.	All	Description	Physical form of the enzyme preparation: liquid, semiliquid or dried product.
20.	All	Purity	Impurities, including elemental and microbiological impurities Analytical methods, validation data, representative batch data (minimum of five batches) are required.
21.	All	Enzyme characterization	Enzyme activity (including method of assay, activity unit), molecular weight of the enzyme and other specific identification techniques. A universally usable test method to define enzyme activity present in the preparation should be submitted. Analytical methods, validation data, representative batch data (minimum of five batches) are required.
22.	All	Analysis of at least five non-consecutive batches of the enzyme concentrate (for enzymes in Class II, at least one of which should have been used for toxicological testing)	e.g. TOS, enzyme activity, protein concentration, impurities, absence of antibiotic inactivating proteins
23.	All	Composition of at least five non-consecutive batches of the product(s) of commerce (enzyme preparation)	e.g. stabilizers, pH adjustment agents, carriers, diluents, preservatives
24.	I (iii), II	Information on carryover of allergens from the fermentation media to the enzyme concentrate	Identification of major food allergens in media components and in the enzyme concentrate
25.	I (iii), II	Evidence of the absence of recombinant DNA and production organisms in the enzyme concentrate or the enzyme commercial product	Requirement applies only to enzymes produced with production organisms that express DNA sequences of concern, such as antibiotic resistance markers.
Assessment of potential allergenicity of the enzyme			
26.	I (iii), II	Comparison of the amino acid sequence of the enzyme with those of known allergens	*In silico* comparison of primary amino acid structure with allergen databases to confirm the absence of sequence homology with known allergenic proteins: • sequence homology (35% of a sliding window of 80 amino acids) • sequence identity in contiguous stretches of eight amino acids in the enzyme sequence All the information resulting from the sequence homology comparison between an expressed enzyme and known allergens should be reported. If any of the identity scores is ≥ 35%, this is considered to indicate significant homology and should be scientifically considered in the context of a safety assessment of enzymes in food.

No.	Class	Information required	Details and rationale
27.	I (iii), II	Proteolysis resistance or digestibility of the enzyme	e.g. studies with simulated gastric fluid
Toxicology			
28.	II	Results of toxicological testing of the enzyme concentrate	Toxicological studies should be conducted to assess whether an ADI should be established: • a 90-day oral toxicity test in a rodent species; and • two short-term genotoxicity tests (mutagenicity and clastogenicity): for gene mutations in bacteria and for micronucleus formation *in vitro*
29.	I (iii), II	Bioinformatic analysis of the amino acid sequence for potential matches with known toxins	Explanation of the analysis and interpretation should be provided.
Dietary exposure assessment			
30.	II	Estimate of dietary exposure to the enzyme preparation calculated on the basis of TOS Various dietary exposure situations might have to be considered, depending on whether they are for: • enzyme preparations added directly to food and not removed; • enzyme preparations added to food but removed from the final product according to good manufacturing practice; or • immobilized enzyme preparations that are in contact with food only during processing.	Express dietary exposure as mg TOS/kg bw per day; provide an explanation of the method used to derive the estimated dietary exposure
31		Additional information and comments	Additional items considered to be helpful in the safety assessment

4. Terms and definitions

Term	Definition
Donor organism	The animal, plant or microorganism that provides the genetic material used to modify the host or recipient organism that will express the enzyme or enzymes of interest. Typically described by genus and species
Host or recipient organism	The animal, plant or microorganism that receives the genetic material from the donor organism. Typically described by genus, species and strain
Production organism or strain	The animal, plant or microorganism used to express the enzyme or enzymes of interest. Typically described by genus, species and strain.
Enzyme	The identity of the specific biologically active protein used to catalyse the reaction of interest. Typically characterized by a specific amino acid sequence and described with EC/IUBMB nomenclature.

Term	Definition
Enzyme concentrate	The product after manufacture (typically from fermentation) and before formulation; it contains the enzyme of interest and other components from the manufacturing process. Is composition is expressed in total organic solids. This is the material usually used in toxicological studies.
Enzyme preparation	Consists of the enzyme concentrate and formulation ingredients; represents the article of commerce used in food production.
Formulation ingredients	Food-grade materials, e.g. stabilizers, pH adjustment agents, carriers, diluents, preservatives, that are added to the enzyme concentrate to make the enzyme preparation.
Total organic solids	Include the enzyme of interest and residues of organic materials, such as proteins, peptides and carbohydrates, derived from the production organism and the manufacturing process, but exclude intentionally added formulation ingredients. % TOS = 100 – (A + W + D) where A = % ash, W = % water and D = % diluents and/or other additives and ingredients.

References

1. General specifications and considerations for enzyme preparations used in food processing. Rome: Food and Agriculture Organization of the United Nations; 2006 (http://www.fao.org/3/a-a0675e.pdf, accessed 24 June, 2019).
2. Evaluation of allergenicity of genetically modified foods. Report of a Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology, 22–25 January 2001. Rome: Food and Agriculture Organization of the United Nations; 2001 (http://www.fao.org/3/a-y0820e.pdf, accessed 9 July 2019).
3. Alinorm 03/34: Joint FAO/WHO Food Standard Programme, Codex Alimentarius Commission, Twenty-Fifth Session. Appendix III, Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants, and Appendix IV, Annex on the assessment of possible allergenicity. Rome: Codex Alimentarius; 2003:47–60.
4. Potential allergenicity of enzymes: change to the number of amino acids in segments used in allergen database searches. In: Eightieth meeting of the Joint FAO/WHO Expert Committee on Food Additives (WHO Technical Report Series; No. 995). Geneva: World Health Organization; 2016.
5. Schmidt DG, Meijer RJ, Slangen CJ, van Beresteijn EC. Raising the pH of the pepsin-catalysed hydrolysis of bovine whey proteins increases the antigenicity of the hydrolysates. Clin Exp Allergy. 1995;25:1007–17.
6. Bannon GA. What makes a food protein an allergen? Curr Allergy Asthma Rep. 2004;4:43–6.
7. Moreno FJ, Mellon FA, Wickham MSJ, Bottrill AR, Mills ENC Stability of the major allergen Brazil nut 2A albumin (Ber e 1) to physiologically relevant in vitro gastrointestinal digestion. FEBS J. 2005;272:341–52.
8. Thomas K, Aalbers M, Bannon GA, Bartels M, Dearman RJ, Esdaile DJ, et al. A multi-laboratory evaluation of a common in vitro pepsin digestion assay protocol used in assessing the safety of novel proteins. Reg Toxicol Pharmacol. 2004;39:87–98.
9. Foods derived from modern biotechnology. Codex Alimentarius. Rome: Food and Agriculture Organization of the United Nations; 2009 (ftp://ftp.fao.org/codex/Publications/Booklets/Biotech/Biotech_2009e.pdf, accessed 25 June 2019).

10. Simulated gastric fluid, TS. In: The National Formulary 9. Rockville (MD): US Pharmacopeia Convention; 2000:2235.

11. Astwood JD, Leach JN, Fuchs RL. Stability of food allergens to digestion in vitro. Nat Biotechnol. 1996;14:1269–73.

12. Vieths S, Reindl J, Müller U, Hoffmann A, Haustein D. Digestibility of peanut and hazelnut allergens investigated by a simple in vitro procedure. Eur Food Res Tech. 1999;209:379–88.

13. Yagami T, Haishima Y, Nakamura A, Osuna H, Ikezawa Z. Digestibility of allergens extracted from natural rubber latex and vegetable foods. J Allergy Clin Immunol. 2000;106(4):752–62.

14. Wal JM. Structure and function of milk allergens. Allergy. 2001;56:35–8.

15. Fu TJ, Abbott UR, Hatzos C. Digestibility of food allergens and nonallergenic proteins in simulated gastric fluid and simulated intestinal fluid – a comparative study. J Agric Food Chem. 2002;50:7154–60.

16. Bøgh KL, Madsen CB. Food allergens: Is there a correlation between stability to digestion and allergencity? Crit Rev Food Sci Nutr. 2015;56:1545–67.

17. Vanhanen M. Exposure, sensitization and allergy to industrial enzymes (People and Work Research Reports 46). Helsinki: Institute of Occupational Health; 2001.

18. Anderson SE, Long C, Dotson GS. Occupational allergy. Eur Med J. 2017;2:65–71

19. Quirce S, Cuevas M, Díez-Gómez M, Fernández-Rivas M, Hinojosa M, González R, et al. Respiratory allergy to Aspergillus-derived enzymes in bakers' asthma. J Allergy Clin Immunol. 1992;90:970–8.

20. Green BJ, Beezhold DH. Industrial fungal enzymes: an occupational allergen perspective. J Allergy. 2011;2011:682574.

21. Olempska-Beer Z, Merker R, Ditto M, DiNovi M. Food-processing enzymes from recombinant microorganisms – a review. Regul Toxicol Pharmacol. 2006;45:144–58.

22. Préstamo G, Manzano P. Peroxidases of selected fruits and vegetables and the possible use of ascorbic acid as an antioxidant. Hort Sci. 1993;28:48–50.

23. Working document on the risk assessment of secondary metabolites of microbial biocontrol agents (OECD Environment, Health and Safety Publications Series on Pesticides No. 98). Paris: organization for Economic Co-operation and Development; 2018 (http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2018)33&doclanguage=en; accessed 21 June 2019).

24. Guidelines for the presentation of data on food enzymes. Opinion expressed on 11 April 1991 (Reports of the Scientific Committee for Food, 27th series). Brussels: Commission of the European Communities; 1992 (http://aei.pitt.edu/40836/1/27th_good.pdf, accessed 25 June 2019).

25. Yu J, Fedorova ND, Montalbano BG, Bhatnagar D, Cleveland TE, Bennett JW, et al. Tight control of mycotoxin biosynthesis gene expression in *Aspergillus flavus* by temperature as revealed by RNA-Seq. FEMS Microbiol Lett. 2011;322:145–9.

26. Inui T, Wang Y, Pro SM, Franzblau SG, Pauli GF. Unbiased evaluation of bioactive secondary metabolites in complex matrices. Fitoterapia. 2012;83:1218–25.

27. Asparaginase from *Aspergillus* oryzae expressed in *Aspergillus oryzae*. In: Sixty-eighth meeting of the Joint FAO/WHO Expert Committee on Food Additives (WHO Technical Report Series;

No. 947). Geneva: World Health Organization; 2008 (https://apps.who.int/iris/bitstream/handle/10665/43870/9789241209472_eng.pdf?sequence=1, accessed 25 June 2019).

Annex 2

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

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

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

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

4. Specifications for identity and purity of food additives (food colours) (Fourth report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as Specifications for identity and purity of food additives, Vol. II. Food colours, Rome, Food and Agriculture Organization of the United Nations, 1963 (out of print).

5. Evaluation of the carcinogenic hazards of food additives (Fifth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 29, 1961; WHO Technical Report Series, No. 220, 1961 (out of print).

6. Evaluation of the toxicity of a number of antimicrobials and antioxidants (Sixth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 31, 1962; WHO Technical Report Series, No. 228, 1962 (out of print).

7. Specifications for the identity and purity of food additives and their toxicological evaluation: emulsifiers, stabilizers, bleaching and maturing agents (Seventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 35, 1964; WHO Technical Report Series, No. 281, 1964 (out of print).

8. Specifications for the identity and purity of food additives and their toxicological evaluation: food colours and some antimicrobials and antioxidants (Eighth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 38, 1965; WHO Technical Report Series, No. 309, 1965 (out of print).

9. Specifications for identity and purity and toxicological evaluation of some antimicrobials and antioxidants. FAO Nutrition Meetings Report Series, No. 38A, 1965; WHO/Food Add/24.65 (out of print).

10. Specifications for identity and purity and toxicological evaluation of food colours. FAO Nutrition Meetings Report Series, No. 38B, 1966; WHO/Food Add/66.25.

11. Specifications for the identity and purity of food additives and their toxicological evaluation: some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases (Ninth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 40, 1966; WHO Technical Report Series, No. 339, 1966 (out of print).

12. Toxicological evaluation of some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases. FAO Nutrition Meetings Report Series, No. 40A, B, C; WHO/Food Add/67.29.

13. Specifications for the identity and purity of food additives and their toxicological evaluation: some emulsifiers and stabilizers and certain other substances (Tenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 43, 1967; WHO Technical Report Series, No. 373, 1967.

14. Specifications for the identity and purity of food additives and their toxicological evaluation: some flavouring substances and non-nutritive sweetening agents (Eleventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 44, 1968; WHO Technical Report Series, No. 383, 1968.

15. Toxicological evaluation of some flavouring substances and non-nutritive sweetening agents. FAO Nutrition Meetings Report Series, No. 44A, 1968; WHO/Food Add/68.33.

16. Specifications and criteria for identity and purity of some flavouring substances and non-nutritive sweetening agents. FAO Nutrition Meetings Report Series, No. 44B, 1969; WHO/Food Add/69.31.

17. Specifications for the identity and purity of food additives and their toxicological evaluation: some antibiotics (Twelfth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 45, 1969; WHO Technical Report Series, No. 430, 1969.

18. Specifications for the identity and purity of some antibiotics. FAO Nutrition Meetings Series, No. 45A, 1969; WHO/Food Add/69.34.

19. Specifications for the identity and purity of food additives and their toxicological evaluation: some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances (Thirteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 46, 1970; WHO Technical Report Series, No. 445, 1970.

20. Toxicological evaluation of some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances. FAO Nutrition Meetings Report Series, No. 46A, 1970; WHO/Food Add/70.36.

21. Specifications for the identity and purity of some food colours, emulsifiers, stabilizers, anticaking agents, and certain other food additives. FAO Nutrition Meetings Report Series, No. 46B, 1970; WHO/Food Add/70.37.

22. Evaluation of food additives: specifications for the identity and purity of food additives and their toxicological evaluation: some extraction solvents and certain other substances; and a review of the technological efficacy of some antimicrobial agents (Fourteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 48, 1971; WHO Technical Report Series, No. 462, 1971.

23. Toxicological evaluation of some extraction solvents and certain other substances. FAO Nutrition Meetings Report Series, No. 48A, 1971; WHO/Food Add/70.39.

24. Specifications for the identity and purity of some extraction solvents and certain other substances. FAO Nutrition Meetings Report Series, No. 48B, 1971; WHO/Food Add/70.40.

25. A review of the technological efficacy of some antimicrobial agents. FAO Nutrition Meetings Report Series, No. 48C, 1971; WHO/Food Add/70.41.

26. Evaluation of food additives: some enzymes, modified starches, and certain other substances: Toxicological evaluations and specifications and a review of the technological efficacy of some

antioxidants (Fifteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 50, 1972; WHO Technical Report Series, No. 488, 1972.

27. Toxicological evaluation of some enzymes, modified starches, and certain other substances. FAO Nutrition Meetings Report Series, No. 50A, 1972; WHO Food Additives Series, No. 1, 1972.

28. Specifications for the identity and purity of some enzymes and certain other substances. FAO Nutrition Meetings Report Series, No. 50B, 1972; WHO Food Additives Series, No. 2, 1972.

29. A review of the technological efficacy of some antioxidants and synergists. FAO Nutrition Meetings Report Series, No. 50C, 1972; WHO Food Additives Series, No. 3, 1972.

30. Evaluation of certain food additives and the contaminants mercury, lead, and cadmium (Sixteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 51, 1972; WHO Technical Report Series, No. 505, 1972, and corrigendum.

31. Evaluation of mercury, lead, cadmium and the food additives amaranth, diethylpyrocarbamate, and octyl gallate. FAO Nutrition Meetings Report Series, No. 51A, 1972; WHO Food Additives Series, No. 4, 1972.

32. Toxicological evaluation of certain food additives with a review of general principles and of specifications (Seventeenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 53, 1974; WHO Technical Report Series, No. 539, 1974, and corrigendum (out of print).

33. Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers, and thickening agents. FAO Nutrition Meetings Report Series, No. 53A, 1974; WHO Food Additives Series, No. 5, 1974.

34. Specifications for identity and purity of thickening agents, anticaking agents, antimicrobials, antioxidants and emulsifiers. FAO Food and Nutrition Paper, No. 4, 1978.

35. Evaluation of certain food additives (Eighteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 54, 1974; WHO Technical Report Series, No. 557, 1974, and corrigendum.

36. Toxicological evaluation of some food colours, enzymes, flavour enhancers, thickening agents, and certain other food additives. FAO Nutrition Meetings Report Series, No. 54A, 1975; WHO Food Additives Series, No. 6, 1975.

37. Specifications for the identity and purity of some food colours, enhancers, thickening agents, and certain food additives. FAO Nutrition Meetings Report Series, No. 54B, 1975; WHO Food Additives Series, No. 7, 1975.

38. Evaluation of certain food additives: some food colours, thickening agents, smoke condensates, and certain other substances (Nineteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 55, 1975; WHO Technical Report Series, No. 576, 1975.

39. Toxicological evaluation of some food colours, thickening agents, and certain other substances. FAO Nutrition Meetings Report Series, No. 55A, 1975; WHO Food Additives Series, No. 8, 1975.

40. Specifications for the identity and purity of certain food additives. FAO Nutrition Meetings Report Series, No. 55B, 1976; WHO Food Additives Series, No. 9, 1976.

41. Evaluation of certain food additives (Twentieth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Food and Nutrition Meetings Series, No. 1, 1976; WHO Technical Report Series, No. 599, 1976.

42. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 10, 1976.

43. Specifications for the identity and purity of some food additives. FAO Food and Nutrition Series, No. 1B, 1977; WHO Food Additives Series, No. 11, 1977.

44. Evaluation of certain food additives (Twenty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 617, 1978.

45. Summary of toxicological data of certain food additives. WHO Food Additives Series, No. 12, 1977.

46. Specifications for identity and purity of some food additives, including antioxidants, food colours, thickeners, and others. FAO Nutrition Meetings Report Series, No. 57, 1977.

47. Evaluation of certain food additives and contaminants (Twenty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 631, 1978.

48. Summary of toxicological data of certain food additives and contaminants. WHO Food Additives Series, No. 13, 1978.

49. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 7, 1978.

50. Evaluation of certain food additives (Twenty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 648, 1980, and corrigenda.

51. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 14, 1980.

52. Specifications for identity and purity of food colours, flavouring agents, and other food additives. FAO Food and Nutrition Paper, No. 12, 1979.

53. Evaluation of certain food additives (Twenty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 653, 1980.

54. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 15, 1980.

55. Specifications for identity and purity of food additives (sweetening agents, emulsifying agents, and other food additives). FAO Food and Nutrition Paper, No. 17, 1980.

56. Evaluation of certain food additives (Twenty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 669, 1981.

57. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 16, 1981.

58. Specifications for identity and purity of food additives (carrier solvents, emulsifiers and stabilizers, enzyme preparations, flavouring agents, food colours, sweetening agents, and other food additives). FAO Food and Nutrition Paper, No. 19, 1981.

59. Evaluation of certain food additives and contaminants (Twenty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 683, 1982.

60. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 17, 1982.

61. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 25, 1982.

62. Evaluation of certain food additives and contaminants (Twenty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 696, 1983, and corrigenda.

63. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 18, 1983.

64. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 28, 1983.

65. Guide to specifications – General notices, general methods, identification tests, test solutions, and other reference materials. FAO Food and Nutrition Paper, No. 5, Rev. 1, 1983.

66. Evaluation of certain food additives and contaminants (Twenty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 710, 1984, and corrigendum.

67. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 19, 1984.

68. Specifications for the identity and purity of food colours. FAO Food and Nutrition Paper, No. 31/1, 1984.

69. Specifications for the identity and purity of food additives. FAO Food and Nutrition Paper, No. 31/2, 1984.

70. Evaluation of certain food additives and contaminants (Twenty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 733, 1986, and corrigendum.

71. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 34, 1986.

72. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 20. Cambridge University Press, 1987.

73. Evaluation of certain food additives and contaminants (Thirtieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 751, 1987.

74. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 21. Cambridge University Press, 1987.

75. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 37, 1986.

76. Principles for the safety assessment of food additives and contaminants in food. WHO Environmental Health Criteria, No. 70. Geneva, World Health Organization, 1987 (out of print). The full text is available electronically at www.who.int/pcs.

77. Evaluation of certain food additives and contaminants (Thirty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 759, 1987, and corrigendum.

78. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 22. Cambridge University Press, 1988.

79. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 38, 1988.

80. Evaluation of certain veterinary drug residues in food (Thirty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 763, 1988.

81. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 23. Cambridge University Press, 1988.

82. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41, 1988.

83. Evaluation of certain food additives and contaminants (Thirty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 776, 1989.

84. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 24. Cambridge University Press, 1989.

85. Evaluation of certain veterinary drug residues in food (Thirty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 788, 1989.

86. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 25, 1990.

87. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/2, 1990.

88. Evaluation of certain food additives and contaminants (Thirty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 789, 1990, and corrigenda.

89. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 26, 1990.

90. Specifications for identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 49, 1990.

91. Evaluation of certain veterinary drug residues in food (Thirty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 799, 1990.

92. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 27, 1991.

93. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/3, 1991.

94. Evaluation of certain food additives and contaminants (Thirty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 806, 1991, and corrigenda.

95. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 28, 1991.

96. Compendium of food additive specifications (Joint FAO/WHO Expert Committee on Food Additives (JECFA)). Combined specifications from 1st through the 37th meetings, 1956–1990. Rome, Food and Agriculture Organization of the United Nations, 1992 (2 volumes).

97. Evaluation of certain veterinary drug residues in food (Thirty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 815, 1991.

98. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 29, 1991.

99. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/4, 1991.

100. Guide to specifications – General notices, general analytical techniques, identification tests, test solutions, and other reference materials. FAO Food and Nutrition Paper, No. 5, Rev. 2, 1991.

101. Evaluation of certain food additives and naturally occurring toxicants (Thirty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 828, 1992.

102. Toxicological evaluation of certain food additives and naturally occurring toxicants. WHO Food Additives Series, No. 30, 1993.

103. Compendium of food additive specifications: addendum 1. FAO Food and Nutrition Paper, No. 52, 1992.

104. Evaluation of certain veterinary drug residues in food (Fortieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 832, 1993.

105. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 31, 1993.

106. Residues of some veterinary drugs in animals and food. FAO Food and Nutrition Paper, No. 41/5, 1993.

107. Evaluation of certain food additives and contaminants (Forty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 837, 1993.

108. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 32, 1993.

109. Compendium of food additive specifications: addendum 2. FAO Food and Nutrition Paper, No. 52, Add. 2, 1993.

110. Evaluation of certain veterinary drug residues in food (Forty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 851, 1995.

111. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 33, 1994.

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

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

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

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

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

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

118. Compendium of food additive specifications: addendum 3. FAO Food and Nutrition Paper, No. 52, Add. 3, 1995.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

214. Evaluation of certain food additives and contaminants (Seventy-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 983, 2013.

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

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

217. Evaluation of certain veterinary drug residues in food (Seventy-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 988, 2014.

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

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

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

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

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

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

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

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

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

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

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

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

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.

236. Compendium of food additive specifications. FAO JECFA Monographs 20, 2017.

237. Safety evaluation of certain food additives. WHO Food Additives Series, No. 75, 2019.

238. 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, 2018.

239. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs 21, 2018.

240. Compendium of food additive specifications. FAO JECFA Monographs 22, 2018.

241. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 76, 2019.

242. 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.

243. Evaluation of certain food additives (Eighty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives) WHO Technical Report Series, No. 1020, 2019.

244. Evaluation of veterinary drug residues in food (Eighty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives) WHO Technical Report Series, No. 1023, 2020.

Annex 3

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
Adenosine 5´-monophosphate deaminase from *Streptomyces murinus*	N	Negative results were observed in genotoxicity tests, and a NOAEL of 500 mg/kg bw per day (equal to 69 mg TOS/kg bw per day) was identified in a 13-week oral toxicity study. Comparison of the dietary exposure estimate of 0.075 mg TOS/kg bw per day with the NOAEL of 69 mg TOS/kg bw per day gives a margin of exposure (MOE) of 920. **The Committee concluded that the AMP deaminase enzyme preparation from S. murinus would not pose a health concern when used in the applications specified, at the levels specified and in accordance with good manufacturing practice.**
D-Allulose 3-epimerase from *Arthrobacter* globiformis expressed in *Escherichia coli*	N	Negative results were observed with D-allulose in genotoxicity tests. A NOAEL of 1100 mg TOS/kg bw per day was identified, the highest dose tested in a short-term (90-day) oral toxicity study in rats. When the dietary exposure estimate for the highest consumers (90th percentile for infants and children) of 0.38 mg TOS/kg bw per day was compared with the NOAEL of 1100 mg TOS/kg bw per day, an MOE of nearly 3000 was calculated. **The Committee established an ADI "not specified" for D-allulose 3-epimerase from *A. globiformis* M30 expressed in *E. coli* K-12 W3110 when the enzyme is used in the applications specified, at the levels specified and in accordance with good manufacturing practice.**
Carbohydrate-derived fulvic acid (CHD-FA)	No[a]	**The Committee concluded that the available data are inadequate for evaluating the safety of CHD-FA.** The Committee assessed the chemical and technical information received and concluded that there was insufficient information to prepare specifications for CHD-FA.
Jagua (genipin-glycine) blue (Jagua blue)	R[b]	The Committee considered that the new toxicological data and additional characterization of the test compound provided adequate information to complete the safety evaluation of Jagua blue. The new 12-month study of rats exposed in utero was conducted for a longer exposure time and at higher doses of Jagua blue, as recommended by the Committee at its 84th meeting. Although no new toxicokinetics study was available, newly developed analytical methods for the dimers provided acceptable characterization of the test article, thus reducing the uncertainty of the safety assessment due to limited biochemical information.

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions
		An ADI of 0–11 mg/kg bw was established by the Committee for Jagua blue, on a blue-polymer basis. This ADI was based on the absence of treatment-related long-term toxicity and of reproductive and developmental toxicity in the 12-month rat dietary study with in-utero exposure, in which the NOAEL was identified as 1127 mg/kg bw per day of the blue polymer, the highest dose tested. The ADI was established by applying an uncertainty factor of 100 to the NOAEL. The Committee noted that the upper end of the high-level dietary exposure estimate for Jagua blue, on a blue-polymer basis, for infants and toddlers of 11.5 mg/kg bw per day is in the region of the upper bound of the ADI. In view of the conservative nature of the dietary exposure assessments, in which it was assumed that all foods contained Jagua blue on a blue-polymer basis at the maximum use level, and because the ADI was based on a NOAEL that was the highest dose tested, the Committee concluded that the estimated dietary exposure to Jagua blue, on a blue-polymer basis, does not represent a health concern.
Lipase from *Mucor javanicus*	N	Negative results were obtained in genotoxicity tests, and no treatment-related adverse effects were seen at the highest dose tested (800 mg TOS/kg bw per day) in a 13-week study of oral toxicity in rats. A comparison of the estimated dietary exposure of 0.84 mg TOS/kg bw per day with the highest dose tested of 800 mg TOS/kg bw per day gives an MOE of at least 900. **The Committee established an ADI "not specified" for the lipase enzyme preparation from *M. javanicus*, used in the applications specified and in accordance with good manufacturing practice.**
Phosphatidylinositol-specific phospholipase C expressed in *Pseudomonas fluorescens* (PI-PLC)	N	Negative results were obtained in genotoxicity tests, and no treatment-related adverse effects were seen with PI-PLC enzyme concentrate at the highest dose tested (1871 mg TOS/kg bw per day) in the 13-week study of oral toxicity in rats. A comparison of the highest estimated dietary exposure of 0.01 mg TOS/kg bw per day with the highest dose tested of 1871 mg TOS/kg bw per day gives an MOE of at least 187 100. **The Committee established an ADI "not specified" for the PI-PLC enzyme preparation expressed in *P. fluorescens*, used in the applications specified and in accordance with good manufacturing practice.**
Riboflavin from *Ashbya gossypii*	No[c]	**Because of time constraints, the assessments of safety and dietary exposure were not completed.**

N: new specifications; R: existing specifications revised
[a] No specifications were prepared. Information is required to prepare specifications (see Annex 4).
[b] The specifications were revised and the tentative status removed.
[c] As the evaluation was postponed, specifications will be published later (see Annex 4).

Food additives assessed only for dietary exposure

Food additive	Conclusions on dietary exposure
Sucrose esters of fatty acids (INS 473) (SEFs) and sucrose oligoesters type I and type II (INS 473a) (SOEs)	At its 49th meeting, the Committee established a group ADI of 0–30 mg/kg bw for SEFs and sucroglycerides on the basis of their potential to induce laxative effects in adult volunteers at doses > 30 mg/kg bw per day, without applying an uncertainty factor. At its 71st meeting, the Committee noted that some of the components of SEFs may be present in significant amounts in SOEs and established a group ADI of 0–30 mg/kg bw for SEFs, SOEs and sucroglycerides. The high dietary exposure estimate of the sum of SEFs and SOEs of 113 mg/kg bw per day for children aged 3–9 years exceeds the group ADI of 0–30 mg/kg bw per day by a factor of about 4. The Committee also noted that the dietary exposure estimates for some other age groups also exceeded the ADI. The Committee noted that the high dietary exposure estimates are conservative, predominantly due to the assumptions that • all foods that could contain SOEs and SEFs do in fact contain these food additives, whereas other food additives with the same functions in foods are available; and • when SEFs or SOEs are used, they are always present at the reported use levels. **Therefore, the Committee considered that more refined dietary exposure estimates should be provided.**

Food additives considered for specifications only

Food additive	Specifications
Magnesium stearate (INS 470(iii))	R[a]
Polyvinyl alcohol (INS 1203)	R[b]
Sorbitan esters of fatty acids (INS 491, INS 492, INS 495)	N[oc]

R: existing specifications revised

[a] For the assay of magnesium, the reference to the ICP-AES method was replaced by a general term, to read "Use a method appropriate to the specified level".

[b] The solubility criterion was changed to "practically insoluble or insoluble in ethanol". For additional remarks, see Annex 4.

[c] No specifications were prepared. Information is required to prepare specifications (see Annex 4).

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

A. Amino acids and related substances

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class I			
Betaine	2265	N	No safety concern
N-Acetyl-glutamate	2269	N	No safety concern
L-Cysteine methyl ester hydrochloride	2270	N	No safety concern
Glutamyl-2-aminobutyric acid	2266	N	No safety concern
Glutamyl-norvaline	2268	N	No safety concern
Glutamyl-norvalyl-glycine	2267	N	No safety concern

B. Phenol and phenol derivatives

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class I			
(±)-Homoeriodictyol sodium salt	2256	N	No safety concern
(±)-Naringenin	2257	N	No safety concern
(2R)-3´,5-Dihydroxy-4´-methoxyflavanone	2258	N	No safety concern
7,8-Dihydroxyflavone	2259	N	No safety concern
(2S)-3´,7-Dihydroxy-8-methyl-4´-methoxyflavan	2260	N	Genotoxicity data for this agent raise concern about potential genotoxicity
(R)-5-Hydroxy-4-(4´-hydroxy-3´-methoxyphenyl)-7-methyl-chroman-2-one	2261	N	No safety concern
3-(3-Hydroxy-4-methoxyphenyl)-1-(2,4,6-trihydroxyphenyl) propan-1-one	2262	N	No safety concern

Flavouring agents considered for specifications only

Food additive	No.	Specifications
4-Hydroxy-2,3-dimethyl-2,4-nonadienoic acid γ-lactone	2002	R[a]
-Caryophyllene oxide	1575	R[b]
2-Acetyl-1-pyrroline	1604	R[c]
(2E,6E/Z,8E)-N-(2-Methylpropyl)-2,6,8-decatrienamide	2077	R[d]
4-Hexen-3-one	1125	R[e]
d-Carvone	380.1	R[f]
2-Pentylfuran	1491	R[g]
3-(2-Furyl)acrolein	1497	R[h]
2-Phenyl-3-(2-furyl)prop-2-enal	1502	R[i]
2-Acetyl-5-methylfuran	1504	R[j]
3-Acetyl-2,5-dimethylfuran	1506	R[k]
4-(2-Furyl)-3-buten-2-one	1511	R[l]
Ethyl 3-(2-furyl) propanoate	1513	R[m]
Phenethyl 2-furoate	1517	R[n]

R: revised

[a] The specific gravity was revised to 0.950–1.000 at 20 °C, and the assay minimum was maintained at 93%, with a change of the secondary component from 1–2% 3,4-dimethyl 5-ketobutanoic acid γ-lactone to 2–3% 3,4-dimethylfuran-2,5-dione.

[b] The melting-point was revised to 55–63 °C and the assay minimum to 95% (sum of isomers). Specifications for the isomeric composition were also established: 84–89% (1*R*,4*R*,6*R*,10*S*) (CAS No. 1139-30-6), 7–9% (1*R*,4*R*,6*S*,10*S*) (CAS No. 60594-22-1), 0.3–2% (1*R*,4*S*,6*S*,10*S*) (CAS No. 103475-43-0) and 1–2% humulene-1,2-epoxide

[c] The assay minimum was revised to 90%, with a secondary component of ≤ 5–6% 5,6-dihydro-2-methyl-3-(4*H*)-pyridinone

[d] The isomeric composition was updated to be 73–80% (2*E*,6*Z*,8*E*), 15–18% (2*E*,6*E*,8*E*), 3–7% (2*E*,6*Z*,8*Z*), 1–2% (2*Z*,6*Z*,8*E*) and 1–2% (2*Z*,6*E*,8*E*).

[e] The assay minimum was set to 95% (sum of isomers), and the specifications for the isomeric composition were established as: 90–95% *trans*-4-hexen-3-one and 1–5% *cis*-4-hexene-3-one.

[f] The refractive index was revised to 1.496–1.502 and the specific gravity to 0.956–0.961.

[g] The refractive index was revised to 1.445–1.451 and the assay minimum to 95%.

[h] The melting point was revised to 42–54 °C.

[i] The physical form and odour were revised.

[j] The specific gravity was revised to 1.065–1.074 and the assay minimum to 95%; the physical form and odour were also revised.

[k] The specific gravity was revised to 1.034–1.048, and the physical form and odour were also revised.

[l] The melting-point was revised to 28–40 °C, and the physical form and odour were also revised.

[m] The physical form and odour were revised, and specifications for the refractive index and the specific gravity were established as 1.455–1.462 and 1.051–1.058, respectively.

[n] The refractive index was revised to 1.540–1.550 and the specific gravity to 1.138–1.150; the physical form and odour were updated.

Annex 4

Secondary components of 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
Aliphatic lactones				
2002	4-Hydroxy-2,3-dimethyl-2,4-nonadienoic acid γ-lactone	93%	3,4-Dimethylfuran-2,5-dione (2–3%)	The SPET value for No. 2002 is 62.5 µg/day, and 3% of this value is 2 µg/day, which is below the class III threshold of toxicological concern.
Aliphatic and aromatic amines and amides				
1604	2-Acetyl-1-pyrroline	> 90%	5,6-Dihydro-2-methyl-3-(4*H*)-pyridinone (5–6%)	The SPET value for No. 1604 is 160 µg/day, and 6% of this value is 10 µg/day, which is below the class III threshold of toxicological concern.
Phenol and phenol derivatives				
2256	(±)-Homoeriodictyol, sodium salt	> 90%	Eriodictyol-7-methyl ether (3–5%); Homoeriodictyol-7-methyl ether (1–2%)	Structurally related (±)-eriodictyol (No. 2172) has been evaluated by the Committee and found to be of no safety concern at estimated dietary exposure when used as a flavouring agent.

Annex 5

Meeting agenda

Food and Agriculture Organization of the United Nations

World Health Organization

89th JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES (JECFA)
1–12 June 2020

Virtual meeting: 12:00–16:00 (Geneva time)

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 the agenda

5. General considerations

   - Update on revised guidance documents for EHC 240
   - Guidance on dose–response assessment and derivation of health-based guidance values
   - Enzyme preparations
   - Genotoxicity studies for chemical substances in food (section 4.5 of EHC 240)

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:
   - Fulvic acid
   - D-Allulose 3-epimerase from *Arthrobacter globiformis* expressed in *Escherichia coli*
   - Lipase from *Mucor javanicus*
   - Jagua blue (genipin–glycine)
   - Phenol and phenol derivatives

- Sucrose esters of fatty acids (INS 473) – exposure only
- Amino acids and related substances
- 5'-Deaminase from *Streptomyces murinus*
- Sucrose oligoesters, type I and type II (INS 473a) – exposure only
- Alicyclic ketones, secondary alcohols and related esters
- Phosphatidyl inositol-specific phospholipase C from a genetically modified strain of *Pseudomonas fluorescens*
- Riboflavin from *Ashbya gossypii*

7.2 Revision of specifications and analytical methods:

- Magnesium stearate (INS 470(iii))
- Polyvinyl alcohol (INS 1203)
- Sorbitan monostearate (INS 491)
- Sorbitan tristearate (INS 492)
- Sorbitan monopalmitate (INS 495)

8. Revision of specifications for certain flavourings.

9. Matters of interest arising from previous Sessions of the Codex Committee on Food Additives

10. Other matters 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)

Further information on these and other WHO publications can be obtained from
WHO Press, World Health Organization ▪ 1211 Geneva 27, Switzerland ▪ www.who.int/bookorders
tel.: +41 22 791 3264; **fax:** +41 22 791 4857; **email:** bookorders@who.int

Evaluation of certain food additives

This report represents the conclusions of a Joint FAO/WHO Expert Committee convened to evaluate the safety of various food additives, including flavouring agents, to identify safety concerns and to prepare specifications for the identity and purity of the food additives.

The first part of the report provides updated guidance documents for the publication Principles and methods for the risk assessment of chemical in food (Environmental Health Criteria 240), specifically on dose–response assessment and derivation of health-based guidance values, evaluation of enzyme preparation and evaluation of the genotoxicity of chemical substances in food. This is followed by summaries of the Committee's evaluations of technical, toxicological and dietary exposure data for seven specific food additives (adenosine 5′-monophosphate deaminase from *Streptomyces murinus,* D-allulose 3-epimerase from *Arthrobacter globiformis* expressed in *Escherichia coli,* carbohydrate-derived fulvic acid, jagua (genipin-glycine) blue, lipase from *Mucor javanicus*, phosphatidylinositol-specific phospholipase C expressed in *Pseudomonas fluorescens* and riboflavin from *Ashbya gossypii*). An exposure assessment was prepared for sucrose esters of fatty acids (INS 473) and sucrose oligoesters type I and type II (INS 473a).

Summaries are also provided of the safety evaluations of two groups of flavouring agents (amino acids and related substances and phenol and phenol derivatives).

Revised specifications were prepared for magnesium stearate (INS 470(iii)), polyvinyl alcohol (INS 1203) and sorbitan esters of fatty acids (INS 491, INS 492, INS 495). The Committee also prepared new and tentative specifications for the new flavouring agents for which there had been a call for data for the meeting and revised the specifications on the basis of the new information.

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 the meeting and the specifications for all the food additives, including flavouring agents.

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