

# Evaluation of certain food additives

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



Food and Agriculture  
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does not necessarily represent the decisions or the stated policy of the World Health Organization*



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Organization**

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

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## List of abbreviations

ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
AMC	anionic methacrylate copolymer
BMC	basic methacrylate copolymer
bw	body weight
CAS	Chemical Abstracts Service
CCFA	Codex Committee on Food Additives
CCFA49	Forty-ninth Session of the Codex Committee on Contaminants in Food Additives
CIFOCoss	FAO/WHO Chronic Individual Food Consumption Database – Summary statistics
CITREM	citric and fatty acid esters of glycerol
CXG	Codex Guideline
CXS	Codex Standard
EFSA	European Food Safety Authority
EHC 240	<i>Environmental Health Criteria 240: Principles and methods for the risk assessment of chemicals in food</i>
EINECS	European Inventory of Existing Commercial Chemical Substances
F	filial generation ( $F_0$ , $F_1$ , $F_2$ , etc.)
FAO	Food and Agriculture Organization of the United Nations
GEWR	Glycerol ester of wood rosin
GLP	good laboratory practice
GRAS	Generally Recognized as Safe
GSFA	General Standard for Food Additives
HPLC	high-performance liquid chromatography
i.p. or IP	intraperitoneal OR intraperitoneal injection
IACM	International Association of Color Manufacturers
IARC	International Agency for Research on Cancer
$IC_{50}$	median inhibitory concentration
INS	International Numbering System for Food Additives
IUPAC	International Union of Pure and Applied Chemistry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
$K_m$	Michaelis constant (affinity)
$LD_{50}$	median lethal dose
LOAEL	lowest-observed-adverse-effect level
LOEL	lowest-observed-effect level
LOQ	limit of quantification
M	male
mADI	microbiological acceptable daily intake



MSDI	maximized survey-derived intake
NCI	National Cancer Institute
NHANES	National Health and Nutrition Examination Survey
NHL	non-Hodgkin lymphoma
NMC	neutral methacrylate copolymer
no. / No.	number
NOAEC	no-observed-adverse-effect concentration
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
NR	not reported
NR	not required
NR	not relevant
NTP	National Toxicology Program
OECD TG	Organisation for Economic Co-operation and Development Test Guideline
OECD	Organisation for Economic Co-operation and Development
<i>P</i>	probability
P90	90th percentile
P95	95th percentile
ppm	parts per million
SCE	sister chromatid exchange
SPET	single-portion exposure technique
TDI	tolerable daily intake
TG	test guideline
TSH	thyroid-stimulating hormone
USA	United States of America
USAID	United States Agency for International Development
USDA	United States Department of Agriculture
USEPA	United States Environmental Protection Agency
USFDA	United States Food and Drug Administration
UV	ultraviolet
UVA	ultraviolet (radiation from about 320–400 nm in wavelength)
v/v	volume per volume
VICH	International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products
WHO	World Health Organization



# 1. Introduction

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) met in Geneva from 12 to 21 June 2018. The meeting was opened by Dr Kazuaki Miyagishima, Director of the Department of Food Safety and Zoonoses of the World Health Organization (WHO), on behalf of WHO Director-General Dr Tedros Adhanom Ghebreyesus. Dr Miyagishima reported that the 2018 World Health Assembly adopted the general programme of work for 2019–2023, and that Codex continues to be a high priority.

Dr Miyagishima welcomed all the delegates. In his opening remarks, he noted the long-standing service of a number of experts, which demonstrates their dedication to JECFA's goals. He also noted the number of new experts, along with the efforts to increase the geographical and gender balance of the Committee, exemplifying JECFA's commitment to knowledge transfer. This was considered particularly welcome as the list of food additives and flavourings awaiting evaluation continues to grow, a sign of the recognition of the quality and importance of JECFA's work.

Dr Miyagishima welcomed JECFA's constant drive to improve its methodology, pointing out that the new methodology for evaluating flavouring agents, agreed on 2 years ago, would be put into practice for the first time by the current Committee. He also informed the Committee that an agreement had been reached between WHO and the International Agency for Research on Cancer (IARC) on new interim standard operating procedures that would streamline the evaluation of compounds and give due recognition of JECFA's primary role in food risk assessments.

Dr Markus Lipp, FAO Joint Secretary, and Dr Angelika Tritscher, WHO Joint Secretary, also welcomed the delegates and thanked them for their hard work before the meeting and their continuing efforts.

## 1.1 Declarations of interests

The Secretariat informed the Committee that all experts participating in the eighty-sixth meeting had completed declaration of interest forms. No conflicts of interest were identified. There were no responses to the public posting of the list of participants for this meeting.

## 1.2 Modification of the agenda

The agenda (see [Annex 4](#)) was modified to include sorbitol syrup in the list of specific food additives based on request of the CCFA to clarify if sorbitol syrup can be covered by the same acceptable daily intake (ADI) as sorbitol.

The agenda (see [Annex 4](#)) was modified to include Corrigenda for specifications monographs.



## 2. General considerations

As a result of the recommendations of the first Joint FAO/WHO Conference on Food Additives (JECFA), held in September 1955 (1), there have been 85 previous meetings of the Committee (Annex 1). The present meeting was convened on the basis of a recommendation made at the eighty-fourth meeting (Annex 1, reference 234).

The tasks before the Committee were to:

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

### 2.1 Report from the Fiftieth Session of the Codex Committee on Food Additives (CCFA)

Dr Yongxiang Fan, Chair of the CCFA, provided the Committee with an update on the work of CCFA since the eighty-fourth meeting of JECFA in 2017 (Annex 1, reference 234).

The Fiftieth Session of CCFA (CCFA50) noted the conclusions of the eighty-fourth meeting of JECFA on the safety of nine food additives. CCFA50 agreed to include gum ghatti (International Numbering System for Food Additives [INS] 419) and tamarind seed polysaccharide (INS 437) in the Codex *General Standard for Food Additives* (CXS 192-1995) (GSFA) Table 3 (“Additives Permitted for Use in Food in General, Unless Otherwise Specified, in Accordance with Good Manufacturing Practice”). CCFA50 noted the conclusions for  $\beta$ -carotene-rich extract from *Dunaliella salina*, Brilliant Blue FCF (INS 133) and Fast Green FCF (INS 143). CCFA50 called on members to provide more data to JECFA to complete the evaluation for Jagua (Genipin–Glycine) Blue, metatartaric acid (INS 353), tannins (oenological tannins) and yeast extracts containing mannoproteins.

CCFA50 finalized work on over 320 provisions for food additives for inclusion in the General Standard on Food Additives (GSFA) and forwarded specifications for the identity and purity of 10 food additives (two new and eight revised specifications) prepared by the eighty-fourth meeting of JECFA for adoption by the forty-first session of the Codex Alimentarius Commission (CAC).

CCFA50 agreed on a revised Priority List of Substances for Evaluation (or re-evaluation) by JECFA, which currently includes 46 food additives and eight flavourings. CCFA50 agreed to amend the circular letter on the Priority List in order to expedite the process of confirming requests and to provide a mechanism for members to confirm the requests without having to attend the in-session working group meetings. CCFA50 also agreed to change the Priority List table by introducing a summary of information about the request such as its basis and possible trade issues.

CCFA50 established an electronic working group that will develop an inventory of data available on nitrates and nitrites with a view to consulting with JECFA and CCFA regarding next steps on the use of these substances,

The Fifty-first Session of CCFA (CCFA51), to be held on 25–29 March 2019 in China, will continue its work on the food additives provisions for inclusion in the GSA, including further alignments of food additive provisions in the Codex commodity standards with the corresponding provisions of the GSA.

## 2.2 Update on activities relevant to JECFA

### 2.2.1 Update of Environmental Health Criteria 240: Principles and methods for the risk assessment of chemicals in food (EHC 240)

The Secretariat informed the Committee about ongoing activities on risk assessment methodology and update of certain chapters of EHC 240. In particular, more detailed guidance on the interpretation and evaluation of genotoxicity of compounds in food, including the interpretation of results of genotoxicity tests, is under development. In addition, Chapter 5 on dose-response assessment, including benchmark dose modelling, and the derivation of health-based guidance values is being updated, as is Chapter 6 on dietary exposure assessment. Guidance for the evaluation of enzyme preparations will also be updated. Final drafts are expected in 2019, following wide stakeholder input including public calls for comments.

## 2.3 Food additive specifications and analytical methods

### 2.3.1 Corrigenda for specifications monographs

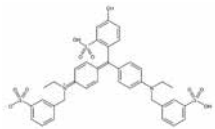
The following requests for corrections, reported to the JECFA Secretariat, were evaluated by the eighty-sixth Committee and found to be necessary (see [Table 1](#)). These corrections, however, will only be made in the online database for specifications.



Table 1  
**Corrections in JECFA reports and monographs on food additives<sup>a</sup>**

Food additive	Original text	New text	Additional information
Calcium disodium ethylenediaminetetraacetate (INS 385) Monograph 1 (2006)	CAS No. 662-33-9	CAS No. 62-33-9	Transcription error
Chlorophyllins, copper complexes sodium and potassium salts (INS 141(ii)) Monograph 5 (2008) Test for "Free ionisable copper"	Accurately weigh about 1 g of the sample and dissolve in 20 ml of arachid oil. . . .	Accurately weigh about 1 g of the sample and mix in 20 mL of arachid oil. . . .	Correction
Curcumin (INS 100(ii)) Monograph 1 (2006)	The criteria for several residual solvents are listed under the heading "Residual solvents" (see Fig. 1).	Acetone: Not more than 30 mg/kg Hexane: Not more than 25 mg/kg Methanol: Not more than 50 mg/kg Ethanol: Not more than 50 mg/kg Isopropanol: Not more than 50 mg/kg Ethyl acetate: Not more than 50 mg/kg	Improves readability  It was unclear whether the criterion "Not more than 50 mg/kg" extended to methanol, ethanol, isopropanol and ethyl acetate.
Ethyl acetoacetate ethyleneglycol ketal JECFA No.: 1969 JECFA 73 (2010)	CAS No. 1648615	CAS No. 6413-10-1	Transcription error
Ethyl 2-methyl pentanoate JECFA No.: 214 JECFA 55 (2000)	CAS No. 28959-02-6	CAS No. 39255-32-8	Wrong CAS number
<i>cis</i> -3-Hexen-1-ol JECFA No.: 315 JECFA 51 (1998)	98.0% (sum of (Z) and (E) isomers, =<92.0% (Z))	98.0% (sum of (Z) and (E) isomers, =>92.0% (Z))	Transcription error
Monosodium <i>L</i> -glutamate (INS 621) Monograph 1 (2006)	CAS No. 142-47-2	CAS No. 6106-04-3	Wrong CAS number
Myrcene JECFA No.: 1327 JECFA 63 (2004)	Specific gravity: 0.789–1.793	Specific gravity: 0.789–0.793	Transcription error
Polyoxyethylene (20) sorbitan monostearat (Polysorbate 60) (INS 435) Monograph 16 (2014)	CAS No. 9005-07-6	CAS No. 9005-67-8	Wrong CAS number
Sodium aluminium silicate (INS 554) Monograph 20 (2017)	Within the assay, the limits for silicon dioxide, aluminium oxide and sodium oxide are expressed "on dried basis".	Within the assay, the limits for silicon dioxide, aluminium oxide and sodium oxide are expressed "on ignited basis".	Transcription error
Silicon dioxide, amorphous (INS 551)	CAS No. 112696-00-8 (hydrated silica)	CAS No. 112926-00-8 (hydrated silica)	Transcription error

Table 1 (continued)

Food additive	Original text	New text	Additional information
Monograph 20 (2017)	Pyrogenic silica is produced in an essentially anhydrous state, whereas the wet process products are obtained as hydrates or contain surface adsorbed water.	Pyrogenic silica is produced in an essentially anhydrous state, whereas the wet process products are obtained as hydrates or contain surface adsorbed water.	Transcription error
Sodium thiosulfate (INS 539) Monograph 1 (2006)	CAS No. 7772-98-7	CAS No. 10102-17-7	CAS No. 7772-98-7 refers to the anhydrous form. The specifications in the monograph refer to the pentahydrate form.
Brown HT and its aluminium lake (FAO JECFA Monographs 19, 82nd meeting, 2016)	Text in Table 1: "Values for synthetic colours for use in performing tests for colouring matters content by spectrophotometry"	See Table 1, below	
Fast Green FCF (FAO JECFA Monographs 19, 82nd meeting, 2016)	Chemical structure in Table 1: "Values for synthetic colours for use in performing tests for colouring matters content by spectrophotometry"		

CAS: Chemical Abstracts Service; INS: International Numbering System for Food Additives; JECFA: Joint FAO/WHO Expert Committee on Food Additives; No.: number  
 \* Bolding and underlining for clarity only. This formatting will not be shown in the online database.

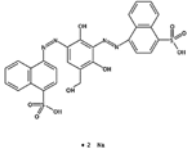
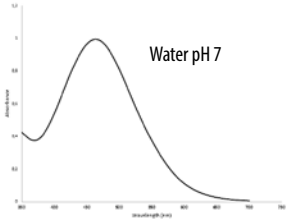
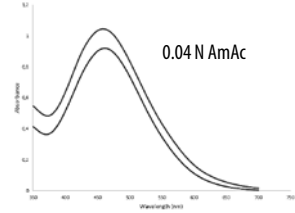
Fig. 1

**Residual solvent criteria for curcumin as displayed in Monograph 1, 2006**

<b>Residual solvents</b> (Vol. 4)	Acetone:	Not more than 30 mg/kg
	Hexane:	Not more than 25 mg/kg
	Methanol:	}
	Ethanol:	
	Isopropanol:	Not more than 50 mg/kg
	Ethyl acetate:	

Table 2

**Replacement of the text for the spectrophotometric data for Brown HT and its aluminium lake originally published in "Table 1. Values for synthetic colours for use in performing tests for Colouring Matters Content by Spectrophotometry" (FAO JECFA Monographs 19, 82<sup>nd</sup> meeting, 2016)**

JECFA name	Sample weight	Structure	Spectral data	Visible absorption spectrum
Brown HT	245.6 mg		Water, pH 7 $\lambda_{\text{max}} = 464$ $A = 0.9957$ Spec abs = 403 $a = 40.3$  Water $\lambda_{\text{max}} = 464$ $A = 0.9804$ Spec abs = 397 $a = 39.7$  0.04 N AmAc $\lambda_{\text{max}} = 461$ $A = 0.9206$ Spec abs = 373 $a = 37.3$	
Brown HT Aluminium Lake	53.3 mg	—	Straight colour (blue) 0.04 N AmAc $\lambda_{\text{max}} = 461$ $A = 0.9206$  Lake (red) 0.04 N AmAc $\lambda_{\text{max}} = 458$ $A = 1.0451$	



## 3. Specific food additives (other than flavouring agents)

### 3.1 Toxicological evaluation, exposure assessment and establishment of specifications

#### 3.1.1 Anionic methacrylate copolymer

##### Explanation

Anionic methacrylate copolymer (AMC; E 1207; International Numbering System Number [INS No.] 1207; Chemical Abstracts Service [CAS] No. 26936-24-3; acrylates copolymers; methyl acrylate, methyl methacrylate, methacrylic acid polymer; methacrylic acid polymer with methyl acrylate and methyl methacrylate) is a copolymer manufactured from the monomers methacrylic acid, methyl methacrylate and methyl acrylate in the molar ratio of 7:3:1.

AMC has been evaluated by the European Food Safety Authority (EFSA) and is approved for use as a food additive in the European Union. Its use in the European Union is restricted to a maximum level of 100 000 mg/kg in solid food supplements, category 17.1 [1].

The Committee considered three different copolymers: basic, anionic and neutral methacrylate copolymers. Each copolymer releases the active ingredients from within their coatings under different physiological conditions in different parts of the digestive tract. AMC is soluble above pH 7 and is used for its taste- and odour-masking properties; as protection from heat, light, moisture and oxidation; and to prevent the fast release of active ingredients once they leave the stomach.

AMC has not previously been evaluated by the Committee. The Committee evaluated the use of AMC as a coating or glazing agent for solid food supplements and foods for special medical purposes when provided in the form of solid food supplements such as capsules, pastilles, tablets, pills, pellets and powders, at levels not exceeding 10%, at the request of the Forty-ninth Session of the CCFA (CCFA49 [2]). AMC is also used in pharmaceuticals.

Toxicological data submitted for the evaluation included distribution studies, acute and short-term toxicity and genotoxicity studies as well as a developmental toxicity study. Limited data were also submitted on the residual monomers. A comprehensive literature search retrieved data on the monomers but no additional studies on AMC. The Committee considered the data on the residual monomers, as well as that for AMC itself, because the monomers are of low molecular weight and therefore likely to be absorbed from the gastrointestinal tract. Due to the low levels of residual monomers present in AMC, the Committee evaluated only absorption, distribution, metabolism and excretion (ADME) data and long-term toxicity and genotoxicity data on the monomers.

The Committee was also aware that AMC contains an oligomer fraction of up to 2%. As the lower end of the molecular weight range for all the constituent oligomers is greater than 1000 Da, with around 75% of the oligomer fraction having a molecular weight between 5000 and 10 000 Da, it is unlikely that the oligomers would be absorbed from the gastrointestinal tract. Therefore, the Committee did not consider the toxicological aspects of the oligomers.

Unless otherwise stated, the test substance used in the distribution and toxicity studies was prepared from an aqueous dispersion with 1.5% emulsifier and then freeze-dried to remove water. In all cases, doses have been expressed as dry weight of AMC.

The Committee evaluated toxicological and exposure data on sodium lauryl sulfate, polysorbate 80 and simethicone, residual components of AMC that can be present in the final product because they are used in the manufacture of the copolymer. The Committee concluded that these residual components did not pose a safety concern at the maximum estimated exposure levels.

### Chemical and technical considerations

AMC is manufactured by emulsion polymerization of the monomers methacrylic acid, methyl methacrylate and methyl acrylate with water-soluble radical initiators. The product is purified by water vapour distillation and filtration to remove residual monomers, excess water, other volatile low molecular weight substances and coagulum.

AMC has a weight-average molecular weight of 280 000 Da and a number-average molecular weight of 77 000 Da.

Although organic solvents are not used in the manufacture of AMC, methanol may be present at a level not exceeding 1000 mg/kg as a result of hydrolysis of esterified carboxyl groups incorporated in the polymer. The copolymer is standardized as a 30% aqueous dispersion with sodium lauryl sulfate (0.3%) and polysorbate 80 (1.2%). Simethicone emulsion is used as an antifoaming agent during the manufacture and is present in the dispersion at not more than 20 mg/kg. The copolymer dispersion may contain residual monomers: methyl acrylate (not more than 1 mg/kg); methyl methacrylate (not more than 3 mg/kg); and methacrylic acid (not more than 1 mg/kg).

### Biochemical aspects

#### AMC

Radiolabelled AMC administered to rats was excreted in the faeces with mean recovery amounting to 92.38% of the total dose after 72 hours and 94.07% ( $\pm 3.42\%$ ) of the total dose 10 days after dosing. Radioactivity was detected at very low levels in the urine of four of the eight treated animals in this group; this may have been due to contamination with faecal matter [3].

## Residual monomers

Methyl methacrylate is rapidly absorbed and distributed following inhalation or oral administration in rats. On the basis of available data, methyl methacrylate is metabolized to methacrylic acid and methanol, which is subsequently converted to carbon dioxide via the tricarboxylic acid cycle in both experimental animals and humans [4, 5, 6].

Methyl acrylate is rapidly absorbed. About half the dose is exhaled as carbon dioxide, while the rest is excreted in the urine in the form of cysteine conjugates and other thioethers [7, 8].

Methacrylic acid is rapidly absorbed after oral or inhalational exposure. After a single oral administration of the sodium salt of methacrylic acid to rats, the maximum concentration in blood was found after 10 minutes. After 60 minutes, no more methacrylic acid was detectable [9].

## Toxicological studies

### AMC

In a good laboratory practice (GLP)-compliant study, a single dose of 2000 mg/kg body weight (bw) of AMC was administered by gavage to rats. Following a 14-day recovery period, there were no abnormal clinical observations, no deaths and no statistically significant differences in body weights between the test animals and historical controls. No abnormalities were observed at necropsy [10].

When gavage doses of 0, 200, 500 or 1500 mg/kg bw per day AMC were administered to rats for 4 weeks, no treatment-related effects were observed. The no-observed-adverse-effect level (NOAEL) was 1500 mg/kg bw per day, the highest dose tested [11]. In a GLP-compliant 26-week oral toxicity study in rats using gavage doses of 0, 200, 500 or 1500 mg/kg bw per day, there were also no treatment-related effects. Some statistically significant findings were observed, but these did not exhibit a dose–response relationship. The NOAEL was 1500 mg/kg bw per day, the highest dose tested [12]. In a 4-week dog study, AMC was administered as copolymer-coated cellulose pellets at doses of the test substance at 0, 100, 200 or 400 mg/kg bw per day. There were no treatment-related effects. The NOAEL was 400 mg/kg bw per day, the highest dose tested [13].

Two reverse mutation assays in bacteria, a mouse lymphoma assay and a chromosomal aberration assay in vitro and an in vivo mouse micronucleus assay all gave negative results. The Committee concluded that AMC was not of concern for genotoxicity.

No long-term toxicity or carcinogenicity studies were available on AMC.

In a GLP-compliant developmental toxicity study, female rats administered AMC at 0 or 1000 mg/kg bw per day by gavage on gestation days 5–19 showed no treatment-related effects. The NOAEL was 1000 mg/kg bw per day, the only dose tested [14].

Studies on cytotoxicity, dermal toxicity, inhalation toxicity, ocular toxicity and phototoxicity produced no effects.

## Residual monomers

### *Methyl methacrylate*

In a long-term toxicity study in rats given methyl methacrylate in drinking-water at 0, 6, 60 or 2000 mg/L (equal to 0, 0.4, 4 and 121 mg/kg bw per day for males and 0, 0.5, 5 and 146 mg/kg bw per day for females, respectively) for 2 years, relative kidney weight increased in females at the highest dose but no treatment-related histopathological effects were observed in any organs or tissues [15]. Based on the results of this study, a tolerable daily intake (TDI) of 1.2 mg/kg bw per day was determined [16].

In long-term toxicity and carcinogenicity studies in mice, rats and hamsters given methyl methacrylate by inhalation, the observed effects were, in general, similar to those reported in the short-term studies but also included inflammation and epithelial hyperplasia of the nasal cavity and degeneration of the olfactory sensory epithelium. There was no evidence of any carcinogenic effects [15].

Bacterial reverse mutation assays with methyl methacrylate gave mostly negative results. Mixed results (i.e. positive, weakly positive or negative) were obtained in in vitro chromosomal aberration and sister chromatid exchange (SCE) assays. One in vitro micronucleus assay was unequivocally negative, whereas a second assay was negative at low concentrations but weakly positive at higher concentrations. Three mouse lymphoma assays for gene mutations were positive. A mouse bone marrow micronucleus assay was negative, but it is not clear if the target tissue was exposed to the test substance. A rat micronucleus assay with exposure by inhalation was positive following 1 day of exposure but negative following 5 days of exposure. These results were judged by the Committee to be inconclusive.

The Committee concluded that there was some evidence of mutagenicity and clastogenicity in vitro but that there was a lack of adequate in vivo tests following up the equivocal in vitro findings.

### *Methyl acrylate*

In a 2-year inhalation study, groups of rats ( $n = 86/\text{sex}$ ) were exposed to methyl acrylate by inhalation at 0, 15, 45 or 135 parts per million (ppm; 0, 53, 158 and 475 mg/m<sup>3</sup>) in air for 6 hours per day on 5 days per week for 2 years. No significant difference in mortality was observed between the groups. The incidence of soft-tissue sarcomas varied considerably between the groups but there was no dose-dependence. No increased frequency of any tumour type in any organ could be related to a carcinogenic effect of the test substance [17].



There are no epidemiological data in humans. The IARC [18] concluded that there was inadequate evidence in experimental animals for carcinogenicity and that methyl acrylate was not classifiable as to its carcinogenicity in humans.

The Committee evaluated the genotoxicity studies on methyl acrylate. Reverse mutation studies in bacteria were negative. A well-performed genotoxicity study on L5178Y mouse lymphoma cells, without exogenous metabolism, produced positive outcomes for gene mutation and chromosomal aberrations but only at cytotoxic concentrations. The *in vivo* study designs have methodological shortcomings. A mouse bone marrow micronucleus assay using intraperitoneal dosing showed an increase in micronuclei induction, but this was limited in magnitude and not dose related. A mouse bone marrow micronucleus assay using oral dosing was negative, but it is not clear if the target tissues were exposed to the test substance. Overall, the Committee concluded that the data were not sufficient to draw conclusions on the genotoxicity of methyl acrylate.

#### *Methacrylic acid*

There were no long-term chronic toxicity/carcinogenicity studies on methacrylic acid. The Committee noted that studies on methyl methacrylate, which is metabolized into methacrylic acid, found no carcinogenicity in mice, rats or hamsters [16].

There are several *in vitro* genotoxicity studies on methacrylic acid, but the most useful data were from negative studies on gene mutations in bacteria. Positive results were obtained in two comet assays and a  $\gamma$ -H2AX assay in human gingival fibroblasts. There were no studies on chromosomal aberrations or gene mutation in mammalian cells and no *in vivo* studies on methacrylic acid. The Committee noted that there were insufficient data to reach a conclusion on the genotoxic potential of methacrylic acid.

#### **Observations in humans**

No human data were available on AMC.

#### **Assessment of dietary exposure**

The Committee evaluated exposure to AMC from its use as a glazing or coating agent in food supplements and foods for special medical purposes. As another major use of AMC is in pharmaceuticals, this use was also evaluated in the exposure assessment. The level of use of AMC is a maximum of 10%.

The Committee evaluated exposure to AMC and its residual monomers, methacrylic acid, methyl methacrylate and methyl acrylate. As the Committee evaluated the toxicology of methacrylic acid, a total exposure to this monomer was estimated from the sum of the methacrylic acid monomer exposure and the methacrylic acid product of methyl methacrylate metabolism.

The exposure assessment included estimates provided by the sponsor and an evaluation by EFSA [1] based on consumption of food supplements and pharmaceuticals. The Committee also estimated exposure based on national food consumption data for food supplements using the concentration proposed by the sponsor. The national consumption data were from the FAO/WHO Chronic Individual Food Consumption Database – Summary statistics (CIFOcOss) and data from Australia and New Zealand submitted to the Committee. A comprehensive literature search was also conducted; no additional studies were identified.

No quantitative estimates of exposure could be determined for foods for special medical purposes. The sponsor indicated that it is not anticipated that foods for special medical purposes would increase exposures above that of food supplements and pharmaceuticals, given the conservative nature of those calculations. In addition, the consumers of foods for special medical purposes will generally be under medical supervision, and exposures for these consumers are not relevant for the general healthy population. This use was not further evaluated by the Committee.

The total monomeric content of AMC is less than 0.01%. This level was used to calculate the exposure to individual monomers based on the ratio of each monomer in the copolymer. The total exposure to methacrylic acid is from the sum of the exposure to methacrylic acid monomers and methacrylic acid from methyl methacrylate with a conversion using molecular weights (Table 3).

The Committee noted that AMC is used in pharmaceuticals. Estimated exposures from this use from the sponsor and EFSA ranged between 8.0 and 13.3 mg/kg bw per day for adults and children. These estimates were within the range of exposures from food supplements. However, the Committee considered that such use should not be taken into account in the assessment of long-term dietary exposure for a healthy population.

## Evaluation

There were no concerns for the toxicity of AMC itself. However, the presence in AMC of the residual monomer methyl acrylate, for which it is not possible to conclude on genotoxic potential, and the insufficient carcinogenicity data for methyl acrylate preclude establishing an ADI for AMC.

The available toxicology data for AMC itself do not give rise to concerns for toxicity. The substance is poorly absorbed and is excreted in the faeces. In short-term and developmental toxicity studies, the NOAELs for AMC range from 400 to 1500 mg/kg bw per day, the highest doses tested. Estimated exposures to AMC range from 2.9 to 43 mg/kg bw per day.

Toxicological data on the residual monomers, apart from the genotoxicity data, do not give rise to concerns when taking into account the low

Table 3

**Summary of range of estimated exposures to AMC, its monomers and total methacrylic acid from uses in food supplements for average and high exposures**

Population group	Range of estimated dietary exposures <sup>a,b</sup>				Total methacrylic acid exposure (µg/kg bw per day) <sup>c</sup>
	AMC (mg/kg bw per day)	Methacrylic acid (µg/kg bw per day)	Methyl methacrylate (µg/kg bw per day)	Methyl acrylate (µg/kg bw per day)	
Adults	3.5–37	0.04–0.4	0.1–0.9	0.2–2.4	0.1–1.2
Children	2.9–43	0.03–0.4	0.1–1.1	0.2–2.8	0.1–1.4

AMC: anionic methacrylate copolymer; bw; body weight

<sup>a</sup> All estimates of exposure are presented as a range from the lowest of the average exposures to the highest of the high exposures. The lower end of each range is the lowest of the estimated average exposures, and the upper end of the range is the highest of the estimated high exposures.

<sup>b</sup> Includes exposures estimates submitted by the sponsor and the European Food Safety Authority [1], and national estimates calculated by the Committee based on a concentration of 100 mg per 1 g dosage unit.

<sup>c</sup> The total methacrylic acid exposure is the sum of the exposure to methacrylic acid monomers and methacrylic acid from methyl methacrylate, with a conversion using molecular weights.

exposures. Genotoxicity data for methyl methacrylate suggest a potential risk for mutagenicity and clastogenicity *in vitro*, and there is a lack of adequate data on genotoxicity *in vivo*. However, in carcinogenicity studies in mice, rats and hamsters with methyl methacrylate given by inhalation, there was no evidence of any carcinogenic effects. In a 2-year study in rats given methyl methacrylate in drinking-water, the NOAEL was 121 mg/kg bw per day; from this NOAEL, a TDI of 1.2 mg/kg bw was derived [16]. Estimated exposures to methyl methacrylate range from 0.1 to 1.1 µg/kg bw per day, which are below the TDI.

Data on methyl acrylate are limited. ADME studies suggest that methyl acrylate is rapidly absorbed and excreted. The genotoxicity data are insufficiently adequate to draw conclusions on the genotoxic potential of methyl acrylate. Although a rat carcinogenicity study on methyl acrylate by the inhalation route was negative, no suitable long-term oral toxicity studies were available to support the safety of methyl acrylate. The Committee was unable to conclude on the safety of methyl acrylate as a residual monomer in AMC. Estimated exposures to methyl acrylate range from 0.2 to 2.8 µg/kg bw per day.

There were insufficient data to reach a conclusion on the genotoxic potential of methacrylic acid, and no long-term carcinogenicity studies were available. The Committee noted that there was evidence that methyl methacrylate is metabolized to methacrylic acid, and therefore the four negative long-term toxicity oral (via drinking-water) and inhalation carcinogenicity studies on methyl methacrylate could be used to support the safety of methacrylic acid. The Committee concluded that the exposure to methacrylic acid from the sum of the levels present in AMC and as a metabolite of methyl methacrylate, ranging from 0.1 to 1.4 µg/kg bw per day, would be unlikely to be a health concern.

The Committee was unable to complete the evaluation of AMC. While the copolymer itself is not a health concern, genotoxicity concerns remain for the residual monomer methyl acrylate.

A toxicological and dietary exposure monograph was prepared.

New specifications and a Chemical and Technical Assessment were prepared. The specifications were made tentative pending the completion of the safety evaluation of AMC.

## References

1. EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS): Scientific Opinion of the safety of anionic methacrylate copolymer for the proposed uses as a food additive. *EFSA J.* 2010;8(7):1656 [25 pp.] doi:10.2903/j.efsa.2010.1656.
2. 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; and Geneva: World Health Organization, Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2017 (REP17/FA).
3. Absorption, distribution and excretion study in the rat following a 13-day administration period. Huntingdon Life Sciences, Eye, Suffolk, England, United Kingdom; 1999. Unpublished study report no. 010/984481 submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
4. Bratt H, Hathway DE. Fate of methyl methacrylate in rats. *Br J Cancer.* 1977;36(1):114–9 [cited in reference 16].
5. Canadian Environmental Protection Act: Priority Substances List assessment report: Methyl methacrylate. Prepared by Health Canada and Environment Canada. Ottawa: Government of Canada; 1993 (Cat. No.: En40-215/10E) [cited in reference 16].
6. Methyl methacrylate – CAS No. 80-62-6. Brussels: European Centre for Ecotoxicology and Toxicology of Chemicals; 1995 (Joint Assessment of Commodity Chemicals No. 30) [cited in reference 16].
7. Vodicka P, Gut I, Frantik E. Effects of inhaled acrylic acid derivatives in rats. *Toxicology.* 1990;65(1–2):209–21.
8. Sapota A. The disposition and metabolism of methyl acrylate in male Wistar albino rats. *Pol J Occup Med Environ Health.* 1993;6(2):185–93.
9. Bereznowski Z, Mariewski M, Somolenski RT. High performance liquid chromatographic determination of methacrylate in blood serum. *Biomed Chromatogr.* 1994;8(1):42–4 [cited in European Commission, 2002].
10. Acute oral toxicity study in the rat. Research Toxicology Centre, Rome, Italy; 1995. Unpublished study report no. 5090/T/211/95 submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
11. Four-week oral toxicity study in rats followed by a 2-week recovery period. Research Toxicology Centre, Rome, Italy; 1998. Unpublished study report no. 861/T/215/98 submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
12. Twenty-six week oral toxicity study in rats followed by a 4-week recovery period. Unpublished study by Research Toxicology Centre, Rome, Italy; 2000. Report no. 7250/T/346/2000 submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.

13. Four-week oral toxicity study in dogs. Unpublished study by Research Toxicology Centre, Rome, Italy; 2004. Report no. 8101 submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
14. Prenatal developmental toxicity study in rats. Unpublished study by Harlan Bioservice for Science, Walsrode, Germany; 2003. Study report no. 20-4-0144-02 submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
15. Borzelleca JF, Larson PS, Hennigar GR, Hluf EG, Crawford EM, Blackwell Smith R. Studies on the chronic oral toxicity of monomeric ethyl acrylate and methyl methacrylate. *Toxicol Appl Pharmacol*. 1964;6:29–36 [cited in reference 16].
16. Methyl methacrylate. Geneva: World Health Organization; 1998 (International Programme on Chemical Safety II. Concise international chemical assessment document 4).
17. Reininghaus W, Koestner A, Klimisch HJ. Chronic toxicity and oncogenicity of inhaled methyl acrylate and *n*-butyl acrylate in Sprague–Dawley rats. *Food Chem Toxicol*. 1991;29(5):329–39.
18. Reevaluation of some organic chemicals, hydrazine and hydrogen peroxide. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 71. Lyon: International Agency for Research on Cancer; 1999.

### 3.1.2 Basic methacrylate copolymer

#### Explanation

Basic methacrylate copolymer (BMC; E 1205; INS No. 1205; CAS No. 24938-16-7; basic butylated methacrylate copolymer; amino methacrylate copolymer; aminoalkyl methacrylate copolymer E; butyl methacrylate, dimethylaminoethyl methacrylate, methyl methacrylate polymer; butyl methacrylate, methyl methacrylate, dimethylaminoethyl methacrylate copolymer) is a cationic copolymer manufactured using the monomers dimethylaminoethyl methacrylate [2-(dimethylamino)ethyl methacrylate], butyl methacrylate [*n*-butyl methacrylate] and methyl methacrylate in the molar ratio of 1:2:1.

BMC has been evaluated by EFSA and is an approved food additive within the European Union where its use is restricted to a maximum level of 100 000 mg/kg in solid food supplements, category 17.1 [1].

The Committee considered three different copolymers: basic, anionic and neutral methacrylate copolymers. Each copolymer releases the active ingredients from within their coatings under different physiological conditions in different parts of the digestive tract. BMC is soluble below pH 5 and is used for its taste- and odour-masking properties; as protection from heat, light, moisture and oxidation; and to prevent the fast release of active ingredients in the stomach.

BMC has not previously been evaluated by the Committee. The Committee evaluated the use of BMC as a coating or glazing agent for solid food supplements and foods for special medical purposes in the form of solid food supplements such as capsules, pastilles, tablets, pills, pellets and powders, at levels not exceeding 10%, at the request of CCFA49 [2]. Another proposed use of BMC is for microencapsulation, which enhances the stability of micronutrients in food

fortification, specifically for populations with nutrient deficiencies. BMC is also used in pharmaceuticals.

Toxicological data submitted for the evaluation included ADME, acute and short-term toxicity and genotoxicity studies as well as a developmental toxicity study. Limited data were also submitted on the residual monomers. A comprehensive literature search retrieved data on the monomers but no additional studies on BMC. The Committee considered the data on the residual monomers because these are of low molecular weight and therefore likely to be absorbed from the gastrointestinal tract. As exposure to monomers from BMC is higher than to AMC and neutral methacrylate copolymer (NMC), the Committee evaluated ADME, short- and long-term toxicity studies, and genotoxicity and reproductive and developmental toxicity data on the monomers.

The Committee was also aware that BMC contains an oligomer fraction of up to 10%. As the lower end of the molecular weight range for all the constituent oligomers is greater than 1000 Da, and around two thirds of the oligomer fraction has a molecular weight of between 4000 and 5000 Da, it is unlikely that the oligomers would be absorbed from the gastrointestinal tract. Therefore, the Committee did not consider the toxicological aspects of the oligomers.

Unless otherwise stated, the test substance used in the distribution and toxicity studies was prepared from an aqueous dispersion and then freeze-dried to remove water. In all cases, doses have been expressed as dry weight of BMC.

The Committee evaluated toxicological and exposure data on 2-propanol, butanol and methanol, the residual components of BMC that can be present in the final products because they are used in the manufacture of the copolymer. The Committee concluded that these residual components do not pose a safety concern at the maximum estimated exposure levels.

### Chemical and technical considerations

BMC is manufactured by a controlled polymerization process using a free radical donor initiator system. After completion of polymerization, the viscous copolymer solution is fed into an extruder to remove solvents and volatile substances, by continuous degassing through vacuum and heating. The solid granules of BMC formed in the extruder can be milled to a powder in which not more than 10% of the particles have a diameter less than 3 µm.

BMC has a weight-average molecular weight of 47 000 Da and number-average molecular weight of 22 000 Da.

During the manufacture of BMC, 2-propanol is added as a production aid. Most of the solvent evaporates during the polymerization and extrusion steps in the manufacture of BMC. The methyl methacrylate integrated in the polymer chain may hydrolyse to methacrylic acid and methanol. The methacrylic acid remains in the polymer chain but methanol is released. Similarly, butyl

methacrylate integrated in the polymer chain may hydrolyse to methacrylate and butanol. BMC may contain 2-propanol (not exceeding 0.5%); butanol (not exceeding 0.5%); and methanol (not exceeding 0.1%). The copolymer may also contain residual monomers: dimethylaminoethyl methacrylate (not exceeding 500 mg/kg); butyl methacrylate (not exceeding 200 mg/kg); and methyl methacrylate (not exceeding 50 mg/kg).

## Biochemical aspects

### BMC

When radiolabelled BMC (purity >98%) was administered to adult rats in two separate studies, the majority was excreted in the faeces within 2–3 days of administration. Levels of radioactivity in the gut returned to normal within 7 days. From the levels of radioactivity in the urine, it can be concluded that less than 0.02% of the radioactivity was absorbed [3].

### Residual monomers

There were no toxicokinetic studies on 2-(dimethylamino)ethyl methacrylate. Studies using simulated saliva or intestinal fluids show that this residual monomer is rapidly hydrolysed with 86–90% degradation [4].

Methyl methacrylate is rapidly absorbed and distributed following inhalation or oral administration to rats. Methyl methacrylate is metabolized to methacrylic acid and methanol, which is subsequently converted to carbon dioxide via the tricarboxylic acid cycle in both experimental animals and humans [5, 6, 7].

Like methyl methacrylate, *n*-butyl methacrylate, is rapidly metabolized by carboxylesterases. Hydrolysis of *n*-butyl methacrylate yields methacrylic acid and *n*-butanol, which are further metabolized, with methacryl CoA, a physiological substrate of the valine pathway [8].

## Toxicological studies

### BMC

In acute toxicity studies in mice, no effects were observed after oral, intraperitoneal and subcutaneous administration of BMC (purity >98%) at 3.0, 2.0 and 1.0 g/kg bw, respectively, although deaths occurred at higher doses via the intraperitoneal and oral routes. In rats, the oral median lethal dose (LD<sub>50</sub>) was greater than 15 g/kg bw. Orally administered lactose granules (125 g) coated in test material (BMC at 5.9 g/kg bw) resulted in deaths in rats, but this mortality was attributed to the amount of the administered dose. When doses of BMC of up to 6 g/kg bw were administered to rats in feed, no adverse effects were observed [9, 10, 11].

Short-term toxicity studies were available in rats and dogs. A 6-month study in rats administered BMC (purity >98%) in feed at 500 or 2000 mg/kg bw per day showed no treatment-related effects [12]. A 28-day study in dogs administered BMC (>98% BMC) in gelatin capsules showed no treatment-related effects at doses of 100–750 mg/kg bw per day [13].

No long-term toxicity or carcinogenicity studies were available on the copolymer itself.

A reverse mutation assay in bacteria, a gene mutation assay in mammalian cells and an in vivo mouse micronucleus assay all produced negative results. The Committee concluded that BMC was not of concern for genotoxicity.

A developmental study in rats showed no treatment-related effects at the only dose tested, 1000 mg/kg bw per day, when administered in feed between gestation days 6 and 16 [14].

Studies of cytotoxicity, dermal toxicity, ocular toxicity and phototoxicity with BMC showed no effects.

#### Residual monomers

##### *2-(Dimethylamino)ethyl methacrylate*

A short-term toxicity study with 2-(dimethylamino)ethyl methacrylate in rats given gavage doses of 0, 100, 200 or 500 mg/kg bw per day for 13 weeks showed no treatment-related adverse effects. The NOAEL was 500 mg/kg bw per day, the highest dose tested [15].

No long-term toxicity studies were available on 2-(dimethylamino)ethyl methacrylate. In genotoxicity studies, 2-(dimethylamino)ethyl methacrylate was negative in two reverse mutation assays in bacteria, apart from one isolated positive response in *Salmonella typhimurium* TA1537 in the absence of metabolic activation (negative in the presence of metabolic activation), and negative in a gene mutation assay in mammalian cells in vitro. The monomer was positive for clastogenicity in two chromosomal aberration tests in mammalian cells in vitro, but negative in a GLP-compliant in vivo mouse erythrocyte micronucleus test conducted according to Organisation for Economic Co-operation and Development (OECD) guideline 474. The Committee concluded that 2-(dimethylamino)ethyl methacrylate does not raise concerns for genotoxicity in vivo.

In a reproductive toxicity study with 2-(dimethylamino)ethyl methacrylate, rats received gavage doses of 0, 40, 200 and 1000 mg/kg per day from 14 days before mating, for 43 days in males and until lactation day 3 in females. There were no effects on reproductive parameters. At the highest dose, maternal toxicity was observed and there were some deaths among dams, with total loss of litters in some dams during the lactation period, reduced pup body weight and reduced pup viability. The NOAEL was 200 mg/kg bw per day [16].



In a developmental toxicity study, 2-(dimethylamino)ethyl methacrylate was administered to female rats by gavage at dose levels of 0, 100, 300 or 600 mg/kg bw per day from gestation days 6 to 19. There were no treatment-related effects on maternal or embryo/fetal parameters. The NOAEL was 600 mg/kg bw per day, the highest dose tested [17]

### *Methyl methacrylate*

In most of the numerous studies on the short-term toxicity of methyl methacrylate in mice, rats, hamsters and dogs, the monomer was administered by inhalation. Most commonly observed effects were decreases in body-weight gain and irritation of the skin, nasal cavity and eye at high concentrations (generally 500 ppm [2050 mg/m<sup>3</sup>]). At even higher concentrations, renal cortical necrosis and tubular degeneration (in rats and mice) and hepatic necrosis (in mice) were also reported [18].

In a long-term toxicity study in rats given methyl methacrylate in drinking-water at 0, 6, 60 or 2000 mg/L (equal to 0, 0.4, 4 and 121 mg/kg bw per day for males and 0, 0.5, 5 and 146 mg/kg bw per day for females, respectively) for 2 years, relative kidney weight in females increased at the highest dose, but no treatment-related histopathological effects were observed in any organ or tissue [19]. Based on the results of this study, a TDI of 1.2 mg/kg bw per day was determined [18].

In long-term toxicity and carcinogenicity studies in mice, rats and hamsters given methyl methacrylate by inhalation, the observed effects were, in general, similar to those reported in the short-term studies but also included inflammation and epithelial hyperplasia of the nasal cavity and degeneration of the olfactory sensory epithelium. There was no evidence of any carcinogenic effects [19].

Bacterial reverse mutation assays with methyl methacrylate gave mostly negative results. Mixed results (i.e. positive, weakly positive or negative) were obtained in in vitro chromosomal aberration and SCE assays. One in vitro micronucleus assay was unequivocally negative, whereas a second assay was negative at low concentrations but weakly positive at higher concentrations. Three mouse lymphoma assays for gene mutations were positive. A mouse bone marrow micronucleus assay was negative, but it is not clear if the target tissue was exposed to the test substance. A rat micronucleus assay with exposure by inhalation was positive following 1 day of exposure but negative following 5 days of exposure. These results were judged by the Committee to be inconclusive. The Committee concluded that there was some evidence of mutagenicity and clastogenicity in vitro. There was a lack of adequate in vivo tests following up the equivocal findings.

There were no conventional reproductive toxicity studies on methyl methacrylate. A dominant lethal study in mice given 100, 1000 or 9000 ppm (410, 4100 and 36 900 mg/m<sup>3</sup>, respectively) of methyl methacrylate by inhalation for 6 hours/day for 5 days showed no effects on fertility [20].

In older studies on mice and rats, developmental effects including decreases in fetal weight and increases in embryo/fetal death and skeletal abnormalities were observed following inhalation of methyl methacrylate at concentrations that were toxic to the dams [21, 22].

In another older developmental toxicity study, pregnant mice were exposed to 1330 ppm (5450 mg/m<sup>3</sup>) of methyl methacrylate for 2 hours, twice daily, during gestation days 6–15. There were no adverse developmental effects [23].

In a developmental toxicity study, rats were given methyl methacrylate by inhalation at concentrations from 9 to 2028 ppm (406–8315 mg/m<sup>3</sup>) for 2 hours daily from gestation days 6 to 15. There were no treatment-related adverse effects. The NOAEL was 8315 mg/m<sup>3</sup> [24].

#### *n*-Butyl methacrylate

In an inhalation study, rats were exposed to *n*-butyl methacrylate at concentrations of 310, 952 or 1891 ppm for 6 hours/day, 5 days/week for 4 weeks. Microscopic examination of the nasal cavities of the male and female rats exposed to 1891 ppm showed slight and localized bilateral degeneration of the olfactory epithelium lining of the dorsal meati. One male and one female rat exposed to 952 ppm had similar changes in the olfactory epithelium. Rats exposed to 310 ppm had no exposure-related microscopic changes in the nasal cavity [25].

No long-term studies were available on *n*-butyl methacrylate.

*n*-Butyl methacrylate tested negative in a range of in vitro and in vivo mutagenicity studies covering all the relevant end-points.

In a developmental toxicity study in rats on four methacrylates, including *n*-butyl methacrylate, animals were exposed by inhalation for 6 hours/day on gestation days 6–20. The exposure concentrations for *n*-butyl methacrylate were 0, 100, 300, 600 or 1200 ppm. Fetal toxicity was evident as decreases in fetal body weight at 600 ppm or greater. These exposure levels of *n*-butyl methacrylate were also maternally toxic. No significant increases in embryo/fetal deaths or fetal malformations were observed [26].

#### Observations in humans

No human data were available on BMC.

#### Assessment of dietary exposure

The Committee evaluated exposure to BMC from its use as a glazing or coating agent in food supplements and foods for special medical purposes as well as

for micronutrient encapsulation for food fortification. Because another major use of BMC is in pharmaceuticals, this use was also evaluated in the exposure assessment. The level of use of BMC in food supplements, pharmaceuticals and foods for special medical purposes is a maximum of 10%. The level of use of BMC for food fortification differs depending on the nutrient or group of nutrients being microencapsulated.

The Committee evaluated exposure to BMC for the copolymer and its monomers, *n*-butyl methacrylate, 2-(dimethylamino)ethyl methacrylate and methyl methacrylate.

The exposure assessment included submitted estimates and an evaluation by EFSA based on consumption of food supplements and pharmaceuticals. The Committee also estimated exposure based on national food consumption data for food supplements using the concentration proposed by the sponsor. National consumption data were from the CIFOCCOss. Data from Australia and New Zealand were submitted to the Committee. A second sponsor provided estimates of dietary exposure from use in micronutrient encapsulation for food fortification. These estimates included exposures for 12 nutrients at a level that met 100% of their respective recommended dietary allowance (RDA). The exposure was estimated for each nutrient individually and also for the sum of all 12 nutrients.

A comprehensive literature search was also conducted; no additional studies were identified.

No quantitative estimates of exposure could be determined for foods for special medical purposes. The sponsor indicated that it is not anticipated that foods for special medical purposes would increase exposures above that of food supplements and pharmaceuticals, given the conservative nature of those calculations. In addition, the consumers of foods for special medical purposes will generally be under medical supervision, and exposures for these consumers are therefore not relevant for the general healthy population. This use was therefore not further considered by the Committee.

The total monomeric content of BMC is less than 0.3%. This level was therefore used to calculate the exposure to total monomers from the copolymer exposure. Estimates of exposure to the individual monomers were based on exposure to total monomers, taking into account the ratio of each individual monomer in the copolymer.

The estimated exposures to BMC and its monomers for adults and children from uses in food supplements and in micronutrient encapsulation for food fortification are shown in [Table 4](#). All estimates of exposure from uses in food supplements are presented as a range from the lowest of the average exposures to the highest of the high exposures. All estimates of dietary exposure from micronutrient encapsulation for food fortification are presented as a range

Table 4

**Summary of the range of estimated exposures to BMC and its monomers for average and high exposures**

Population group	Source of exposure	Range of estimated dietary exposures <sup>a,b</sup>			
		Copolymer exposure (mg/kg bw per day)	Monomer exposure (µg/kg bw per day)		
			<i>n</i> -Butyl methacrylate	2-(Dimethylamino) ethyl methacrylate	Methyl methacrylate
Adults	Food supplements	3.5–37	2.6–28	5.2–56	2.6–28
	Micronutrient encapsulation for food fortification <sup>c</sup>	0.03–45	0.02–33	0.04–67	0.02–33
	All sources	3.5–82	2.6–61	5.2–120	2.6–61
Children	Food supplements	2.9–43	2.2–32	4.4–64	2.2–32
	Micronutrient encapsulation for food fortification <sup>c</sup>	0.05–92	0.04–69	0.1–140	0.04–69
	All sources	3.0–135	2.2–100	4.4–200	2.2–100

BMC: butyl methacrylate; bw: body weight

<sup>a</sup> All estimates of exposure are presented as a range from the lowest of the average exposures to the highest of the high exposures. The lower end of each range is the lowest of the estimated mean exposures, and the upper end of each range is the highest of the estimated high exposures.

<sup>b</sup> Includes exposure estimates submitted by the sponsor and the European Food Safety Authority [1] and national estimates calculated by the Committee. Based on a concentration of 100 mg per 1 g dosage unit.

<sup>c</sup> Based on meeting 100% of the United States/Canadian recommended dietary allowance. The lower end of each range is lowest exposure for a single nutrient and the upper end of each range is the sum of exposure from 12 nutrients.

from the lowest exposure for a single nutrient to the exposure for the sum of the 12 nutrients assessed.

The Committee noted that the exposures estimated by the sponsor were to provide a presumed worst case for the purpose of the safety evaluation. The Committee also noted that national estimates of exposure would be required for the evaluation of safety based on their own nutrient reference values, fortification needs and food consumption patterns.

Estimates of exposure to BMC and its monomers from all sources combined (food supplements and micronutrient encapsulation for food fortification) are also shown in Table 4. The Committee noted that the upper end of the range representing high exposures from all sources is a worst case estimate, and unlikely in terms of actual long-term exposure to BMC.

The Committee noted that BMC is used in pharmaceuticals. Estimated exposures from this use from the sponsor and EFSA ranged between 8.0 and 13.3 mg/kg bw per day for adults and children. These estimates were within the range of exposures from food supplements. However, the Committee considered that such use should not be taken into account in the assessment of long-term dietary exposure for a healthy population.

## Evaluation

The Committee concluded that the use of BMC that complies with the specifications established at the current meeting is not a safety concern when the food additive is used as a coating or glazing agent for solid food supplements and for foods for special medical purposes and micronutrient encapsulation for food fortification.

An ADI “not specified” was established for BMC.

The available toxicology data for BMC do not give rise to concerns for toxicity. The substance is poorly absorbed and is excreted in the faeces. In short-term and developmental toxicity studies, the NOAELs for BMC range from 750 to 2000 mg/kg bw per day, the highest doses tested.

Toxicological data on the residual monomers do not give rise to concerns when taking into account the low exposures. 2-(Dimethylamino) ethyl methacrylate and *n*-butyl methacrylate do not give rise to concerns for genotoxicity. Long-term, reproductive and developmental toxicity studies do not suggest a risk to health at the estimated exposure levels. Genotoxicity data for methyl methacrylate suggest a potential for mutagenicity and clastogenicity in vitro, but there is a lack of adequate in vivo genotoxicity data. However, in carcinogenicity studies in mice, rats and hamsters given methyl methacrylate by inhalation, there was no evidence of any carcinogenic effects. In a 2-year study in rats given methyl methacrylate in drinking-water, the NOAEL was 121 mg/kg bw per day; from this NOAEL, a TDI of 1.2 mg/kg bw per day was derived [18]. Estimated exposures to methyl methacrylate range from 2.2 to 100 µg/kg bw per day, which are below the TDI.

A toxicological and dietary exposure monograph was prepared.

New specifications and a Chemical and Technical Assessment were prepared.

## References

1. EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS); Scientific Opinion on the use of basic methacrylate copolymer as a food additive. *EFSA J.* 2010;8(2):1513 [23 pp.] doi:10.2903/j.efsa.2010.1513.
2. 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; and Geneva: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2017 (REP17/FA).
3. [<sup>14</sup>C]-Eudragit-E-100: The excretion and tissue distribution of radioactivity after oral administration to rats. Report no. 79/RGC024/278. Life Science Research, Stock, Essex, England, United Kingdom; 1979. Unpublished study report. Submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.

4. 2-Dimethylaminoethyl methacrylate: Basic toxicokinetics. EC no.: 220-688-8. CAS no.: 2867-47-2. Helsinki: European Chemicals Agency; 2015a (<https://echa.europa.eu/registration-dossier/-/registered-dossier/14994/7/2/2>, accessed 6 November 2015).
5. Bratt H, Hathway DE. Fate of methyl methacrylate in rats. *Br J Cancer*. 1977;36(1):114–9 [cited in reference 18].
6. Canadian Environmental Protection Act: Priority Substances List assessment report for methyl methacrylate. Prepared by Health Canada and Environment Canada. Ottawa: Government of Canada; 1993.(Cat. no.: En40-215/10E) [cited in reference 18].
7. ECETOC. Methyl methacrylate – CAS no. 80-62-6. Brussels: European Centre for Ecotoxicology and Toxicology of Chemicals; 1995 (Joint Assessment of Commodity Chemicals No. 30) [cited in reference 18].
8. IUCLID [database]. Butyl methacrylate (97-88-1) [2000 CD-ROM edition] [48 pp.] European Commission; 2010 (<http://esis.jrc.ec.europa.eu/>).
9. Determination of acute oral toxicity in rats. Unpublished study report by Battelle-Institute, Frankfurt am Main, Germany; 1966. Submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
10. The acute toxicity of preparation 2787 on oral administration to rats. Unpublished study report. Hamburg, Germany: Laboratory for Pharmacology and Toxicology; 1970. Submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
11. Acute toxicity of Eudragit E. Unpublished study by Department of Pharmacology, Nagasaki University, Nagasaki, Japan; 1970. Submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
12. Six-month toxicity of 2787 B in Sprague-Dawley rats with administration in the feedstuff. Report no. 100692 by Laboratory for Pharmacology and Toxicology, Hamburg, Germany; 1973. Unpublished study report submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
13. Twenty-eight day oral toxicity study of Eudragit E PO in the dog. Unpublished study report no. 20-2-0145-02 by Harlan Bioservice for Science, Walsrode, Germany; 2003. Submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
14. Experiments to determine the effect of 2577G and 2697 on pregnant rats and their foetuses. Unpublished study by Laboratory for Pharmacology and Toxicology, Hamburg, Germany; 1968. Submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
15. 2-Dimethylaminoethyl methacrylate. EC no.: 220-688-8. CAS no.: 2867-47-2. Repeated dose toxicity: oral. Helsinki: European Chemicals Agency; 2015b (<https://echa.europa.eu/registration-dossier/-/registered-dossier/14994/7/6/2>, accessed 20 June 2018).
16. 2-Dimethylaminoethyl methacrylate: toxicity to reproduction. EC no.: 220-688-8. CAS no. 2867-47-2. Helsinki: European Chemicals Agency; 2015c (<https://echa.europa.eu/registration-dossier/-/registered-dossier/14994/7/9/2>, accessed 20 June 2018).
17. 2-Dimethylaminoethyl methacrylate. EC no.: 220-688-8. CAS no.: 2867-47-2. Developmental toxicity / teratogenicity. Helsinki: European Chemicals Agency; 2015d (<https://echa.europa.eu/registration-dossier/-/registered-dossier/14994/7/9/3>, accessed 20 June 2018).
18. Methyl methacrylate. Concise international chemical assessment document 4. Geneva: World Health Organization; 1998 (International Programme on Chemical Safety, Concise International Chemical Assessment Document No. 4).

19. Borzelleca JF, Larson PS, Hennigar GR Jr, Huf EG, Crawford EM, Smith RB Jr. Studies on the chronic oral toxicity of monomeric ethyl acrylate and methyl methacrylate. *Toxicol Appl Pharmacol.* 1964;6:29–36 [cited in reference 18].
20. Anderson D, Hodge MCE. Methyl methacrylate monomer: Dominant lethal study in the mouse. ICI, Macclesfield, Cheshire, England, United Kingdom; 1976. Report no. CTL/P/295 [cited in reference 18].
21. Hodge MCE, Palmer S. Methylmethacrylate monomer teratogenicity studies in the rat. Unpublished study by Rohm and Haas Company, Philadelphia, Pennsylvania, USA, to the US Environmental Protection Agency, Washington (DC), USA, under TSCA Section 8(d), July 1979; 1977 [cited in reference 18].
22. Nicholas CA, Lawrence WH, Autian J. Embryotoxicity and fetotoxicity from maternal inhalation of methyl methacrylate monomer in rats. *Toxicol Appl Pharmacol.* 1979;50(3):451–8 [cited in reference 18].
23. McLaughlin RE, Reger SI, Barkalow JA, Allen MS, Diffazio CA. Methyl methacrylate: A study of teratogenicity and fetal toxicity of the vapor in the mouse. *J Bone Joint Surg Am.* 1978;60(3):355–8 [cited in reference 18].
24. Solomon HM, McLaughlin JE, Swenson RE, Hagan JV, Wanner FJ, O'Hara GP et al. Methyl methacrylate: Inhalation developmental toxicity study in rats. *Teratology.* 1993;48(2):115–25.
25. ECHA. Butyl methacrylate. EC no.: 202-615-1. CAS no.: 97-88-1. Repeated dose toxicity: Inhalation. Helsinki: European Chemicals Agency; 2018 (<https://echa.europa.eu/registration-dossier/-/registered-dossier/15151/7/6/3>, accessed 20 June 2018).
26. Saillenfait AM, Bonnet P, Gallissot F, Peltier A, Fabriès JF. Developmental toxicities of methacrylic acid, ethyl methacrylate, *n*-butyl methacrylate, and allyl methacrylate in rats following inhalation exposure. *Toxicol Sci.* 1999;50(1):136–45 [cited in reference 25].

### 3.1.3 Erythrosine

#### Explanation

Erythrosine (INS No. 127; CAS No. 16423-68-0) is a xanthene dye permitted as a food colour in China, the European Union, the USA and other regions. It is used for colouring foods including baked goods, breakfast cereals, confectionery products, dairy products, decorations for baking, dressings and sauces, dried fruit, frostings and icings, frozen breakfast foods, frozen treats, hot beverages, juice drinks and processed foods (fish, meat and egg products).

The Committee previously evaluated the safety of erythrosine at its eighth, thirteenth, eighteenth, twenty-eighth, thirtieth, thirty-third and thirty-seventh meetings ([Annex 1](#), references 8, 19, 35, 66, 73, 83 and 94) and for dietary exposure at its fifty-third meeting ([Annex 1](#), reference 143). Toxicological monographs or monograph addenda were published after the thirteenth, eighteenth, twenty-eighth, thirtieth, thirty-third and thirty-seventh meetings ([Annex 1](#), references 20, 36, 67, 74, 84 and 95). At its eighteenth meeting the Committee allocated an ADI of 0–2.5 mg/kg bw. This ADI was reduced at the twenty-eighth meeting to 0–1.25 mg/kg bw and made temporary following observations that erythrosine

produced effects on thyroid function in short-term toxicity studies in rats and that, in long-term toxicity studies, male rats receiving 4% erythrosine in the diet developed benign thyroid tumours.

At the thirtieth meeting the Committee reduced the temporary ADI to 0–0.6 mg/kg bw, based on biochemical effects of erythrosine on thyroid hormone metabolism and regulation in rats. The Committee at the thirtieth meeting requested further data from pharmacokinetic studies relating the amount of erythrosine absorbed to the amount ingested to enable the establishment of a correlation between blood/tissue erythrosine levels and effects on the thyroid. At the thirty-third meeting the Committee further reduced the temporary ADI to 0–0.05 mg/kg bw. This ADI was based on a NOAEL from a study showing slightly increased thyroid-stimulating hormone (TSH) responsiveness in humans ingesting erythrosine at 60 mg per person per day (equivalent to 1 mg/kg bw per day) for 14 days, and applying an uncertainty factor<sup>2</sup> of 20. The Committee again requested the pharmacokinetic studies required by the previous Committee. At its thirty-seventh meeting, the Committee re-evaluated previously reviewed studies that had since been published and newer studies on thyroid physiology in rats. The Committee concluded that the thyroid tumours in male rats previously reported in long-term toxicity studies were secondary to thyroid hormone changes and species-specific sensitivity.

In view of the differences in thyroid physiology between humans and rats, the Committee based its evaluation on the human data and allocated an ADI of 0–0.1 mg/kg bw on the NOAEL of 60 mg per person per day from the 14-day study in human subjects (equivalent to 1 mg/kg bw per day), with application of an uncertainty factor of 10.

At the present meeting, the Committee re-evaluated erythrosine at the request of CCFA49 [1].

A toxicological dossier that included new studies on genotoxicity, reproductive and developmental toxicity, neurological effects and hypersensitivity was submitted. A comprehensive literature search conducted in PubMed retrieved three additional studies relevant to the present evaluation. The Committee also considered studies evaluated at previous meetings.

### Chemical and technical considerations

Erythrosine consists of the disodium salt of 2-(2,4,5,7-tetraiodo-6-oxido-3-oxoxanthen-9-yl)benzoate monohydrate and subsidiary colouring matters. Sodium chloride and/or sodium sulfate are the principal uncoloured components. Erythrosine is manufactured by iodination of fluorescein, the condensation product of resorcinol and phthalic anhydride. Impurities include unreacted starting materials ( $\leq 0.4\%$ ), subsidiary colouring matters except fluorescein

<sup>2</sup> The previous Committee used the term “safety factor”.



( $\leq 4\%$ ), fluorescein ( $\leq 20$  mg/kg), inorganic iodides ( $\leq 0.1\%$ ), lead ( $\leq 2$  mg/kg) and zinc ( $\leq 50$  mg/kg).

### Biochemical aspects

Erythrosine is poorly absorbed and mainly excreted unchanged in rat faeces.

Following oral administration of radiolabelled erythrosine, less than 1% of the dose was excreted in the urine. Blood and plasma radioactivity reached maximum levels by 1 hour, while liver and kidney levels peaked after 4–12 hours; no radioactivity was detectable in the brain or pituitary. No erythrosine was accumulated in the thyroid or other tissues [2].

No additional metabolic or kinetic studies have become available since the previous evaluation by the Committee.

### Toxicological studies

Erythrosine has low oral acute toxicity in mice, rats, gerbils and rabbits.

A short-term toxicity study with erythrosine showed pigment deposition in renal tubules in female rats at the 2% dietary level and in males at all but the lowest dietary levels, in a dose-related manner. The NOAEL was 0.25% in the diet (equal to 160 mg/kg bw per day) [3]. No compound-related effects were observed in other short-term toxicity studies in rats, gerbils, dogs and pigs. No additional short-term toxicity studies have become available since the previous evaluation by the Committee.

Several long-term oral toxicity studies showed no compound-related increases in tumour incidences in mice, rats and gerbils. In one long-term study of oral toxicity [4], mice showed decreased body weights at 3.0% erythrosine in the diet. The NOAEL was 1.0% erythrosine in the diet (equal to 1474 mg/kg bw per day). Body-weight decreases were also observed in a rat study [5]. The NOAEL was 1% erythrosine in the diet (equivalent to 500 mg/kg bw per day).

Two long-term feeding studies with erythrosine found an increase in the incidence of thyroid follicular cell adenomas in male rats [6, 7]. The previous Committee considered the occurrence of thyroid follicular tumours in rats secondary to hormonal effects based on results from studies on thyroid function and morphology. Another study indicated that erythrosine promoted the development of thyroid follicular tumours in partially thyroidectomized rats, but not in non-thyroidectomized rats [8]. The present Committee noted that the rat is not considered a suitable model for potential effects on the thyroid in humans [9].

A large number of *in vitro* and *in vivo* genotoxicity tests have been conducted on erythrosine. The Committee confirmed that the overall weight of evidence indicates that erythrosine is not genotoxic.

Two short-term oral toxicity studies indicated that erythrosine may affect testicular function in mice. Abdel-Aziz et al. [10] observed decreased epididymal sperm counts and sperm motility at dose levels of 68 and 136 mg/kg bw per day and a significant increase in the incidence of abnormal sperm head morphology at doses of 680 and 1360 mg/kg bw per day. Vivekanandhi et al. [11] observed decreases in sperm motility and sperm counts and increases in sperm abnormalities, with a lowest-observed-adverse-effect level (LOAEL) of 64 mg/kg bw per day. No effects on sperm or on fertility were observed in rats in long-term toxicity studies and a multigeneration study conducted with higher doses administered via the diet [6, 7, 12].

No developmental toxicity was reported in rats.

In a newly available reproductive toxicity study in mice, administration of erythrosine at dietary concentrations of 0, 0.005%, 0.015% or 0.045% (equivalent to 0, 7.5, 22.5 and 67.5 mg/kg bw per day, respectively) resulted in no adverse effects on reproductive parameters or functional developmental parameters in offspring. Significant changes in some measures of exploratory behaviour and motor activity were reported in high-dose dams and their offspring [13]. Motor activity and brain serotonin levels in rat single-dose and repeated-dose studies were inconsistent [14, 15]. In view of the lack of consistency in the data, the poor oral absorption of erythrosine, the evidence that erythrosine does not penetrate the blood–brain barrier and the lack of evidence of behavioural toxicity in a rat study with dose levels equivalent to up to 1000 mg/kg bw per day [16], the Committee concluded that the findings in the Tanaka [13] and Dalal & Poddar [14, 15] studies did not provide robust evidence of behavioural effects and could not be used in the risk assessment.

### Observations in humans

Studies in human volunteers showed increased blood iodine levels but no changes in thyroid hormone levels in repeated-dose studies of up to 25 mg/day for 3 weeks or single-dose studies of up to 80 mg. A study with 30 healthy male volunteers found that erythrosine slightly increased thyroid-stimulating hormone (TSH) responsiveness to thyrotropin-releasing hormone (TRH), but there were no effects on other thyroid hormone parameters at the highest dose level of 200 mg per person per day for 14 days [17]. No effect was observed at 60 mg per person per day. At its thirty-third meeting, the Committee concluded that the NOAEL was 60 mg per person per day (equivalent to 1 mg/kg bw per day), and the present Committee concurred.

### Assessment of dietary exposure

The Committee previously evaluated dietary exposure to erythrosine at its fifty-third meeting, but significant changes in methodologies for the exposure

Table 5  
**Summary of the range of estimates of dietary exposure for erythrosine**

Source of estimates	Population	Range of estimated dietary exposures (mg/kg bw per day)	
		Means	High percentile (P90 and P95) <sup>a</sup>
Analytical levels <sup>b</sup>	Children	0.00–0.01	0.00–0.09
	Adolescents	0.00–0.02	0.00–0.04
	Adults	0.00–0.03	0.00–0.05
Maximum use levels <sup>c</sup>	Toddlers	0–0.2	0–0.4
	Children	0–0.1	0–0.1
	Adolescents	0–0.1	0–0.1
	Adults	0–0.1	0–0.2
	Elderly adults	0–0.1	0–0.1

bw: body weight; P90: 90th percentile; P95: 95th percentile

<sup>a</sup> The upper bound of the range is the maximum of the 90th and 95th percentiles.

<sup>b</sup> Studies from the Republic of Korea [18] and the USA [19, 20] for consumers only.

<sup>c</sup> From published national studies [21, 22]) and the Committee's assessment based on consumption data from CIFOCoSs for 36 countries.

assessments precluded comparisons between the previous and the current assessments.

A comprehensive literature search retrieved five relevant studies other than those submitted by the sponsor. The Committee reviewed published estimates of dietary exposure to erythrosine conducted in several countries. The Committee also conducted a conservative assessment using consumption data from 36 countries from the CIFOCoSs database and Codex maximum use levels. A summary of the dietary exposure estimates is provided in [Table 5](#).

Dietary exposure estimates based on individual consumption data and maximum use levels range from 0 to 0.4 mg/kg bw. Because erythrosine has been in use for many years, national estimates based on analytical data were also available to the Committee. National exposure estimates based on analytical data range from 0.00 to 0.05 mg/kg bw per day for adults and from 0.00 to 0.09 mg/kg bw per day for children, considering consumers only.

The Committee also noted that exposure through pharmaceuticals was previously estimated to occur at up to approximately 0.1 mg/kg bw per day in specific populations, generally over a short period of time. However, the Committee considered that such exposure should not be taken into account in the assessment of exposure to erythrosine as a food additive when looking at long-term exposure in a healthy population.

The Committee considered the approach based on analytical data to be more realistic for preparing long-term dietary exposure estimates than the approach based on maximum use levels. Because the scenarios available from the national studies examined consumers only, the Committee concluded that the

highest estimate of 0.09 mg/kg bw per day for children should be considered in the safety assessment of erythrosine.

## Evaluation

The evidence newly available at this meeting indicates that there are no concerns with respect to genotoxicity and reproductive and developmental toxicity of erythrosine. The previously established ADI of 0–0.1 mg/kg bw is based on a NOAEL of 60 mg per person per day (equivalent to 1 mg/kg bw per day for a 60 kg person) identified in a human study, with a default uncertainty factor of 10. In this study [17], minimal effects on thyroid function were observed at 200 mg per person per day (equivalent to 3.3 mg/kg bw per day). Effects in experimental animals were observed at doses at least 60-fold higher than the NOAEL in this human study; these effects supported the use of the human data as the basis for the ADI.

The Committee concluded that the new data that have become available since the previous evaluation do not give reason to revise the ADI and confirmed the previous ADI of 0–0.1 mg/kg bw.

The Committee noted that the dietary exposure estimate for erythrosine of 0.09 mg/kg bw per day (95th percentile for children) was close to the upper bound of the ADI. Given that this estimate of exposure is for children and it is a high percentile for consumers only, such a level is unlikely to occur every day over a lifetime. Therefore, the Committee concluded that dietary exposures to erythrosine for all age groups do not present a safety concern.

A consolidated toxicological and dietary exposure monograph was prepared.

At the present meeting, the existing specifications for erythrosine were revised. High-performance liquid chromatographic (HPLC) methods were added for determining subsidiary colouring matters and organic compounds other than colouring matters. The method of assay was changed to visible spectrophotometry, and spectrophotometric data were provided for the colour dissolved in water.

A Chemical and Technical Assessment was 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; and Geneva: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2017 (REP17/FA).
2. Obrist J, LeVan L, Puhl RJ, Duan RJ. Final report: Metabolism of FD&C Red No. 3 in rats, study no. 6145–100. Unpublished report by Hazleton Laboratories America, Inc., 1986. Submitted to WHO by the Certified Color Manufacturers Association, Inc., Washington, DC, USA.

3. Butterworth KR, Gaunt IF, Grasso P, Gangolli SD. Acute and short-term toxicity studies on erythrosine BS in rodents. *Food Cosmet Toxicol.* 1976;14(6):525–31.
4. Borzelleca JF, Hallagen JB. Lifetime toxicity/carcinogenicity study of FD & C Red No. 3 (erythrosine) in mice. *Food Chem Toxicol.* 1987;25(10):735–7.
5. Hansen WH, Davis KJ, Graham SL, Perry CH, Jacobson KH. Long-term toxicity studies of erythrosine. II. Effects on haematology and thyrosine and protein-bound iodine in rats. *Food Cosmet Toxicol.* 1973;11(4):535–45.
6. Brewer L, Jefferson ND, Blair M, Thorstenson JB, Nair KPC, Richter WR et al. Long-term dietary toxicity/carcinogenicity study in rats. FDC Red No. 3. Unpublished report no. 410–002 from International Research and Development Corporation, Mattawan, MI, USA; 1981. Submitted to WHO by the Certified Color Manufacturers Association Inc., Washington, DC, USA.
7. Borzelleca JF, Capen CC, Hallagen JB. Lifetime toxicity/carcinogenicity study of FD & C Red No. 3 (erythrosine) in rats. *Food Chem Toxicol.* 1987;25(10):723–33.
8. Hiasa Y, Ohshima M, Kitahori Y, Konishi N, Shimoyama T, Sakaguchi Y et al. The promoting effects of food dyes, erythrosine (Red 3) and Rose Bengal B (Red 105), on thyroid tumors in partially thyroidectomized *N*-bis(2-hydroxypropyl)-nitrosamine-treated rats. *Jpn J Cancer Res.* 1988;79(3):314–9.
9. Part 1: IPCS framework for analysing the relevance of a cancer mode of action for humans and case-studies. Geneva: World Health Organization, International Programme on Chemical Safety; 2007; pp. 1–125. Harmonization Project Document No. 4.
10. Abdel-Aziz AH, Shouman SA, Attia AS, Saad SF. A study on the reproductive toxicity of erythrosine in male mice. *Pharmacol Res.* 1997;35(5):457–62.
11. Vivekanandhi J, Devi CPA, Jayaraman K, Raghavan L. Effect of erythrosine on testicular function of mice. *Toxicol Int.* 2006;13(2):119–25.
12. Aldridge D, Schardein JL, Blair M, Goldenthal EI, Benson BW. FD & C Red No. 3. Three generation reproduction study. Unpublished report no. 410–013 from International Research and Development Corporation, Mattawan, MI, USA; 1981. Submitted to WHO by the Certified Color Manufacturers Association, Inc., Washington, DC, USA.
13. Tanaka T. Reproductive and neurobehavioural toxicity study of erythrosine administered to mice in the diet. *Food Chem Toxicol.* 2001;39(5):447–54.
14. Dalal A, Poddar MK. Short-term erythrosine B-induced inhibition of the brain regional serotonergic activity suppresses motor activity (exploratory behavior) of young adult mammals. *Pharmacol Biochem Behav.* 2009;92(4):574–82.
15. Dalal A, Poddar MK. Involvement of high plasma corticosterone status and activation of brain regional serotonin metabolism in long-term erythrosine-induced rearing motor hyper activity in young adult male rats. *Toxicol Mech Methods.* 2010;20(6):287–97.
16. Vorhees CV, Butcher RE, Brunner RL, Wootten V, Sobotka TJ. A developmental toxicity and psychotoxicity evaluation of FD and C Red Dye No. 3 (erythrosine) in rats. *Arch Toxicol.* 1983;53(4):253–64.
17. Gardner DF, Utiger RD, Schwartz SL, Witorsch P, Meyers B, Braverman LE et al. Effects of oral erythrosine on thyroid function in normal men. *Toxicol Appl Pharmacol.* 1987;91(3):299–304.
18. Ha MS, Ha SD, Choi SH, Bae DH. Exposure assessment of synthetic colours approved in Korea. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* 2013;30(4):643–53.

19. Doell DL, Folmer DE, Lee HS, Butts KM, Carberry SE. Exposure estimate for FD&C colour additives for the US population. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* 2016;33(5):782–97.
20. Bastaki M, Farrell T, Bhusari S, Bi X, Scrafford C. Estimated daily intake and safety of FD&C food-colour additives in the US population. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* 2017;34(6):891–904.
21. Gilsenan MB, Gibney MJ. Assessment of the influence of energy under-reporting on intake estimates of four food additives. *Food Addit Contam.* 2004;21(3):195–203.
22. Final assessment report. Application A603. Red 3 erythrosine in food colouring preparations. Canberra: Food Standards Australia New Zealand; 2010.

### 3.1.4 Indigotine

#### Explanation

Indigotine (INS No. 132) consists of a mixture of disodium 3,3'-dioxo-[delta2,2'-biindoline]-5,5'-disulfonate (the principal component; CAS No. 860-22-0), disodium 3,3'-dioxo-[delta2,2'-biindoline]-5,7'-disulfonate (an isomer) and subsidiary colouring matters. Indigotine is used in China, the European Union, Japan, the USA and other regions. It is used for colouring foods including blueberry bagels, breakfast cereals, cakes and cupcakes, candies including chocolate, chewing gum, dairy products, decorations for baking, frozen treats, sauces and seasonings.

The Committee evaluated the safety of indigotine at its thirteenth and eighteenth meeting ([Annex 1](#), references 19 and 35). At its eighteenth meeting, the Committee established an ADI of 0–5 mg/kg bw based on a NOAEL of 500 mg/kg bw per day (1% in the diet) in a chronic dietary toxicity study in rats [1], and prepared specifications for indigotine ([Annex 1](#), reference 35). At its seventy-third meeting, the Committee revised the specifications ([Annex 1](#), references 202).

Indigotine was on the agenda for re-evaluation of safety and revision of specifications at the request of CCFA49 [2].

A toxicological dossier was submitted summarizing the available toxicity data, together with relevant study reports and publications. A comprehensive literature search retrieved eight other relevant studies.

#### Chemical and technical considerations

Indigotine is an indigoid dye. Indigotine consists of a mixture of disodium 3,3'-dioxo-[delta2,2'-biindoline]-5,5'-disulfonate, disodium 3,3'-dioxo-[delta2,2'-biindoline]-5,7'-disulfonate (an isomer) and subsidiary colouring matters. Sodium chloride and/or sodium sulfate are the principal uncoloured components. Indigotine is manufactured by heating indigo in the presence of sulfuric acid. The indigo (or indigo paste) is manufactured by the fusion of

*N*-phenylglycine (prepared from aniline and formaldehyde) in a molten mixture of sodamide and sodium and potassium hydroxides under ammonia pressure. It is isolated and subjected to purification procedures prior to sulfonation. Impurities include unreacted starting materials ( $\leq 0.5\%$ ), subsidiary colouring matters ( $\leq 1\%$ ), unsulfonated primary aromatic amines ( $\leq 0.01\%$  calculated as aniline) and lead ( $\leq 2$  mg/kg).

### Biochemical aspects

Although indigotine was poorly absorbed from the gastrointestinal tract in rats, one of its metabolites, 5-sulfoanthranilic acid, formed through microbial fermentation, was absorbed to a greater extent. After administration of a single oral dose of 50 mg/rat, unchanged indigotine, isatin-5-sulfonic acid and 5-sulfoanthranilic acid were identified in urine (0.53%, 0.63% and 0.28% of the dose, respectively). The metabolites isatin-5-sulfonic acid and 5-sulfoanthranilic acid were also identified in bile [3]. After *in vitro* incubation of indigotine with caecal microflora of rats, four unidentified metabolites were found [4].

*In vitro*, indigotine significantly inhibited the CYP2A6 monooxygenase activity in a noncompetitive manner, with a median inhibitory concentration ( $IC_{50}$ ) of 0.05 mmol/L [5, 6].

### Toxicological studies

Indigotine has a low acute toxicity. No adverse effects were seen in pigs fed indigotine (purity  $>85\%$ ) at dose levels of 0, 150, 450 and 1350 mg/kg bw per day for 90 days [7].

When mice were fed diets containing 0.2%, 0.4%, 0.8% or 1.6% indigotine (purity  $\geq 85\%$ ) for 80 weeks, the only effect seen was a slight anaemia in animals at 0.8% or 1.6% [8]. The previous Committee concluded that indigotine was not carcinogenic and that the “no-untoward-effect level was 0.4% of the diet equivalent to an intake of approximately 550 mg/kg/day” (Annex 1, references 35 and 36).

In a 2-year combined toxicity and carcinogenicity study, mice were fed indigotine (purity 93%; 7% volatile matter) at dietary concentrations of 0, 0.5%, 1.5% or 5.0% (equal to 0, 825, 2477 and 8259 mg/kg bw per day, respectively). A moderate incidence of blue-green discoloration of the gastrointestinal tract, with occasional discoloration of the liver, gall-bladder and urine, was observed at all doses. There was no evidence of carcinogenicity. The NOAEL was 5% in the diet (equal to 8259 mg/kg bw per day), the highest dose tested [9].

In a long-term combined toxicity and carcinogenicity study, which included an *in utero* phase, indigotine (purity 93%, 7% volatile matter) was fed to rats at dietary levels of 0, 0.5%, 1.0% or 2.0% (equal to 0, 304, 632 and 1282 mg/kg bw per day, respectively). Treatment began approximately 2 months prior

to mating, and the long-term phase was initiated after random selection of the F<sub>1</sub> animals [10]. No consistent substance-related adverse effects were noted, with the exception of statistically significant increases in incidences of malignant mammary gland tumours and gliomas in males, but not females, at the highest dose. The incidence of mammary gland tumours (carcinomas/adenocarcinomas) in high-dose males was 5.9% (3/51 compared with 0/114 control animals). The incidence of gliomas in high-dose males was 9.9% (7/71) compared with 2.9% in controls (4/140 animals). No increase was seen in the low and mid dose groups (incidence 2.9%, 2/70 animals) or in female rats.

The previous Committee noted the statistically significant increase in incidence of malignant mammary gland tumours and gliomas in male rats at the highest dose level of 1282 mg/kg bw per day. In the absence of any indications for genotoxicity, the Committee concluded that the NOAEL in this study was 632 mg/kg bw per day.

Rats fed a diet containing 1% indigotine (equivalent to 500 mg/kg bw per day) for 2 years showed no treatment-related pathological signs, and fewer malignant and benign tumours than the controls; they also survived for longer than the controls [11]. When indigotine was fed to groups of rats at dietary levels of 0, 0.5%, 1.0%, 2.0% or 5.0% for 2 years, the only effect seen was a reduced growth in males at 2.0% and 5.0% [1]. The NOAEL was 1% in the diet, equivalent to 500 mg/kg bw per day.

Indigotine tested negative in a series of bacterial mutagenicity assays as well as in *in vitro* mammalian cell chromosomal aberration assays and a number of comet assays. Indigotine was also not genotoxic in *in vivo* micronucleus tests in mice and rats and a comet assay in mice. The Committee concluded that indigotine does not raise any concerns with respect to genotoxicity.

No reproductive or developmental toxicity was observed in one 3-generation rat study (doses up to 250 mg/kg bw); two 2-generation rat studies (doses up to 250 or 500 mg/kg bw); one rat teratogenicity study (doses up to 250 mg/kg bw); and two rabbit teratogenicity studies (doses up to 250 mg/kg bw).

### Assessment of dietary exposure

Dietary exposure to indigotine has not been previously reviewed by the Committee.

A comprehensive literature search retrieved five relevant studies other than those submitted by the sponsor. The Committee reviewed published estimates of dietary exposure to indigotine conducted in several countries and regions. A summary of these estimates, showing the results from mean and high percentile calculations, is provided in [Table 6](#).

As indigotine has provisions in 51 food categories in the General Standard on Food Additives (GSFA), an exposure assessment based on maximum use



Table 6

**Summary of the range of estimates of dietary exposure for indigotine**

Source of estimates	Population	Range of estimated dietary exposures (mg/kg bw per day)	
		Mean	High percentile (P90 and P95) <sup>a</sup>
National <sup>b</sup>	Children	0.00–0.01	0.00–0.09
	Children	0.00–0.03	0.0–0.3
	Adolescents	0.00–0.01	0.00–0.03
	Adults	0.00–0.01	0.00–0.02
Europe <sup>c</sup>	Toddlers	0.0–0.2	0.3–0.8
	Children	0.0–0.1	0.2–0.8
	Adolescents	0.0–0.1	0.1–0.5
	Adults	0.0–0.4	0.1–0.3
	Elderly adults	0.0–0.03	0.1–0.2

bw: body weight; P90: 90th percentile; P95: 95th percentile

<sup>a</sup> The upper bound of the range is the maximum of the 90th and 95th percentiles.

<sup>b</sup> Data from Australia [12]; Kuwait [13]; the Netherlands [14]; the Republic of Korea [15]; and the USA [16, 17].

<sup>c</sup> Based on consumption data from 17 European countries [18].

levels was considered by the Committee to be unrealistic. Moreover, analytical and reported use levels for the main contributing food categories were far below the maximum use levels, reinforcing the Committee's decision not to consider estimates based on maximum use levels for the exposure assessment.

Because indigotine has been authorized for use for many years, estimates based on analytical data were available and were considered by the Committee to be more appropriate for determining long-term dietary exposure estimates. Exposure estimates based on analytical data range from 0.0 to 0.8 mg/kg bw per day for adults, adolescents and children at the 95th percentile. Because of the conservative assumptions in high percentile exposure estimates, the Committee concluded that an estimate of 0.8 mg/kg bw per day for children and toddlers should be considered in the safety assessment for indigotine.

## Evaluation

The Committee noted that indigotine is poorly absorbed from the gastrointestinal tract, has a low acute toxicity, is not genotoxic and does not show any potential for reproductive or developmental toxicity. The previous Committee identified a NOAEL of 500 mg/kg bw per day from a 2-year rat study of 1% indigotine in the diet and established an ADI of 0–5 mg/kg bw. The current Committee considered the new data that had become available since the previous evaluation as well as previously evaluated studies. In one long-term toxicity study, slight anaemia was observed in mice fed diets with 0.8% or 1.6% indigotine. In another long-term toxicity study, body-weight gain was reduced in male rats at 2.0% and 5.0%

indigotine in the diet. In a third long-term toxicity study, increased incidences of malignant mammary gland tumours and gliomas were observed in male rats at 1282 mg/kg bw per day but not at 304 and 632 mg/kg bw per day.

The Committee concluded that there are no reasons to revise the ADI and confirmed the ADI of 0–5 mg/kg bw.

The Committee noted that the conservative dietary exposure estimate of 0.8 mg/kg bw per day (95th percentile for children and toddlers) is less than the upper limit of the ADI of 0–5 mg/kg bw established for indigotine. The Committee concluded that dietary exposure to indigotine for all age groups does not present a health concern.

A consolidated toxicological and dietary exposure monograph was prepared.

The existing specifications for indigotine were revised. HPLC methods were added for determining subsidiary colouring matters and organic compounds other than colouring matters. The method of assay was changed to visible spectrophotometry, and spectrophotometric data were provided for the colour dissolved in water.

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

## References

1. Hansen WH, Fitzhugh OG, Nelson AA, Davis KJ. Chronic toxicity of two food colors, brilliant blue FCF and indigotine. *Toxicol Appl Pharmacol.* 1966;8(1):29–36.
2. 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 and Geneva: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2017 (REP17/FA).
3. Lethco EJ, Webb JM. The fate of FD&C Blue No. 2 in rats. *J Pharmacol Exp Ther.* 1966;154(2):384–9.
4. Singh S, Das M, Khanna SK. Bio-metabolism of green S and indigo carmine through caecal microflora of rats. *Biochem Biophys Res Commun.* 1993;195(1):490–96.
5. Kuno N, Mizutani T. Influence of synthetic and natural food dyes on activities of CYP2A6, UGT1A6, and UGT2B7. *J Toxicol Environ Health A.* 2005;68(16):1431–44.
6. Mizutani T. Toxicity of xanthene food dyes by inhibition of human drug-metabolizing enzymes in a noncompetitive manner. *J Environ Public Health.* 2009;953952:1–9. doi:10.1155/2009/953952.
7. Gaunt IF, Kiss IS, Grasso P, Gangolli SD. Short-term toxicity study on indigo carmine in the pig. *Food Cosmet Toxicol.* 1969;7(1):17–24.
8. Hooson J, Gaunt IF, Kiss IS, Grasso P, Butterworth KR. Long-term toxicity of indigo carmine in mice. *Food Cosmet Toxicol.* 1975;13(2):167–76.
9. Borzelleca JF, Hogan GK. Chronic toxicity/carcinogenicity study of FD & C Blue No. 2 in mice. *Food Chem Toxicol.* 1985;23(8):719–22.

10. Borzelleca JF, Hogan GK, Koestner A. Chronic toxicity/carcinogenicity study of FD & C Blue No. 2 in rats. *Food Chem Toxicol.* 1985;23(6):551–8.
11. Oettel H, Frohberg H, Nothdurft H, Wilhelm G. [Testing of some synthetic dyes for their suitability as food coloring agents]. *Arch Toxikol.* 1965;21(1):9–29 (in German).
12. Survey of added colours in foods available in Australia. Study of concentration in foods including dietary exposure assessment and risk characterisation. Canberra: Food Standards Australia New Zealand; 2008.
13. Husain A, Sawaya W, Al-Omair A, Al-Zenki S, Al-Amiri H, Ahmed N et al. Estimates of dietary exposure of children to artificial food colours in Kuwait. *Food Addit Contam.* 2006;23(3):245–51.
14. Kist-van Holthe JK, Altenburg TM, Bolakhrif S, El Hamdi L, Man MW, Tu J et al. Consumption of artificial food colourings by school children in the Netherlands. *Adv Pediatr Res.* 2015;2(5). doi:10.12715/apr.2015.2.5.
15. Ha MS, Ha SD, Choi SH, Bae DH. Exposure assessment of synthetic colours approved in Korea. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* 2013;30(4):643–53.
16. Doell DL, Folmer DE, Lee HS, Butts KM, Carberry KM. Exposure estimate for FD&C colour additives for the US population. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* 2016;33(5):782–97.
17. Bastaki M, Farrell T, Bhusari S, Bi X, Scrafford C. Estimated daily intake and safety of FD&C food-colour additives in the US population. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* 2017;34(6):891–904.
18. Scientific Opinion on the re-evaluation of Indigo Carmine (E 132) as a food additive. EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS). *EFSA J.* 2014;12(7):3768. doi:10.2903/j.efsa.2014.3768.

### 3.1.5 Lutein and lutein esters from *Tagetes erecta* and zeaxanthin (synthetic)

#### Explanation

Lutein esters from *Tagetes erecta* (INS No. 161b(iii)) and lutein from *Tagetes erecta* (INS No. 161b(i)) are used as food colouring agents and nutrient supplements in a wide range of baked goods and baking mixes, beverages and beverage bases, breakfast cereals, chewing gum, dairy product analogues, egg products, fats and oils, frozen dairy desserts and mixes, gravies and sauces, soft and hard candy, infant and toddler foods, milk products, processed fruits and fruit juices, soups and soup mixes at levels ranging from 2 to 330 mg/kg.

Zeaxanthin (INS No. 161h(i)) is used as a nutritional supplement and colour in a wide range of foods such as baked goods, beverages, chewing gum, egg products, fats and oils, gravies and sauces, hard and soft candy, infant and toddler foods (other than infant formula), milk products, processed fruits and fruit juices, soups and soup mixes at levels ranging from 0.5 to 70 mg/kg.

Lutein esters contain lutein (all-*E*,3*R*,3'*R*,6'*R*)- $\beta$ , $\epsilon$ -carotene-3,3'-diol, which is a naturally occurring oxygenated xanthophyll pigment and a macular carotenoid. Lutein occurs with its isomeric xanthophyll, zeaxanthin, in many

foods but particularly in vegetables and fruit. Lutein occurs either esterified to fatty acids or in a non-esterified “free” form. Studies show that *meso*-zeaxanthin, a structurally related xanthophyll of lutein, and zeaxanthin may originate from foodstuffs that are sources of these xanthophylls, rather than being derived from the bioconversion of retinal lutein [1]. Extracts containing xanthophylls (free and/or esterified) are used as colours and as nutritional supplements in a wide range of applications.

At the thirty-first meeting, the Committee prepared tentative specifications for xanthophylls obtained from *Tagetes erecta* petals, but no toxicological evaluation was performed (Annex 1, reference 77). Xanthophyll preparations (*Tagetes* extract) containing lutein esters at low concentrations were evaluated at the fifty-fifth and fifty-seventh meetings (Annex 1, references 149 and 154). The tentative specifications (Annex 1, reference 151) were subsequently superseded by full specifications (Annex 1, reference 156). At the sixty-third meeting, the Committee evaluated biochemical data and the results of toxicological and human studies on *Tagetes* preparations with a high content of non-esterified lutein (>80%) and established a group ADI of 0–2 mg/kg bw per day for lutein from *Tagetes erecta* and synthetic zeaxanthin (Annex 1, reference 173). At the seventy-ninth meeting, the Committee evaluated the results of new toxicity studies on preparations with a higher content of xanthophyll esters (>60%), identified as “lutein esters from *Tagetes erecta*” (Annex 1, reference 220). A temporary ADI “not specified” for lutein esters from *Tagetes erecta* was established; the ADI was made temporary because the specifications for lutein esters from *Tagetes erecta* were tentative.

At the eighty-second meeting, the Committee evaluated additional information on manufacturing and composition of lutein esters from *Tagetes erecta* that permitted the adoption of final specifications and the removal of the temporary designation (Annex 1, reference 230). An ADI “not specified” for lutein esters from *Tagetes erecta* was established. The Committee was unable to consider establishing a group ADI “not specified” for lutein esters from *Tagetes erecta*, lutein from *Tagetes erecta* and synthetic zeaxanthin and related xanthophylls, and recommended that this be taken up at a future meeting.

At the present meeting, newly available data for lutein, zeaxanthin and *meso*-zeaxanthin were submitted by the sponsor. A comprehensive literature search retrieved two other studies. The Committee assessed available toxicity studies for re-evaluation of safety, dietary exposure and specifications to consider establishing a group ADI “not specified” for lutein and lutein esters from *Tagetes erecta* and zeaxanthin (synthetic).

## Chemical and technical considerations

Lutein, lutein esters (including lutein dipalmitate) and zeaxanthin are members of a group of pigments known as xanthophylls; they have no provitamin A activity.

*Tagetes* extract (INS No. 161b(ii)) is obtained by hexane extraction of dried petals of marigold (*Tagetes erecta* L.), with subsequent removal of the solvent. The major colouring principles are lutein (all-*E*,3*R*,3'*R*,6'*R*- $\beta$ , $\epsilon$ -carotene-3,3'-diol) and lutein dipalmitate (helenien;  $\beta$ , $\epsilon$ -carotene-3,3'-diol dipalmitate). Hydroxy derivatives of carotenes together with other oxy derivatives, such as epoxides, may also be present in *Tagetes* extract. The product may contain fats, oils and waxes that occur naturally in the plant material. The articles of commerce are usually further formulated to standardize the colour content or to obtain water-soluble or dispersible products.

Lutein esters from *Tagetes erecta* (INS No. 161b(iii)) is a purified extract obtained from marigold (*Tagetes erecta* L.) oleoresin, which is extracted using organic solvents. The preparation contains lutein esters, of which lutein dipalmitate accounts for the major part; a smaller proportion of zeaxanthin esters is also present. The balance of the extract is made up of naturally occurring waxes.

Lutein from *Tagetes erecta* (INS No. 161b(i)) is a purified extract obtained from marigold (*Tagetes erecta* L.) oleoresin, which is extracted using organic solvents. The final product, after saponification and crystallization, contains lutein as the major component and a smaller proportion of zeaxanthin.

Zeaxanthin (synthetic) (INS No. 161h(i)) is the synthetic all-*trans* isomer of zeaxanthin (3*R*,3'*R*- $\beta$ , $\beta$ -carotene-3,3'-diol) produced by the Wittig reaction from raw materials that are commonly used in the production of other carotenoids with application in foods. Minor quantities of *cis*-zeaxanthins and by-products 12-apozeaxanthinal, parasiloxanthin, diatoxanthin and triphenyl phosphine oxide may be present in the final product.

## Biochemical aspects

The ADME of lutein and lutein esters from *Tagetes erecta* was extensively described in the monographs at the sixty-third and seventy-ninth meetings ([Annex 1](#), references 174 and 221). The absorption of lutein involves emulsification by bile, followed by lipolysis by pancreatic lipases into the micellar fraction for absorption by intestinal cells. Absorption of xanthophylls from esters requires hydrolysis. Hydrolysis is an efficient process as esterified lutein is not normally found in human serum. These processes have been shown to be influenced by food matrices, for example, dietary fibres reduce absorption whereas dietary fats promote absorption due to the hydrophobic nature of these xanthophylls. The bioavailability of lutein following administration of lutein esters has been shown to be equivalent to the administration of free lutein.

Sheshappa et al. [2] investigated the influence of food matrices in lutein-deficient rats. Lutein levels in plasma, liver and eyes were higher than in the controls when animals were administered lutein-mixed micelles containing either 3% fat, phosphatidyl choline (PC) or lyso-phosphatidyl choline (lysoPC). In contrast, the administration of lutein-mixed micelles containing pectin and mixed xanthophylls resulted in lutein levels that were lower than in the controls. Evans et al. [3] found that in humans given a single dose of 20 mg of lutein in a starch-based matrix or cross-linked to an alginate matrix, lutein from the starch-based product was better absorbed than lutein from the alginate one.

In humans, dietary supplements of lutein (containing 5% zeaxanthin) at 10 mg/day for 6 months increased the mean serum level of lutein from a baseline value of 210 to 1000 nmol/L and of zeaxanthin from 56 to 95 nmol/L [4].

Albert et al. [5] observed that monkeys fed a xanthophyll-free diet supplemented with either pure lutein or pure zeaxanthin for 12–92 weeks formed 3'-dehydrolutein in plasma. In addition, two 3'-dehydrolutein diastereomers, (3*R*,6'*S*)- and (3*R*,6'*R*)-3'-dehydrolutein, were present in nearly equimolar concentrations. The authors considered these findings to be comparable to those in human plasma after dietary supplementation with either lutein or zeaxanthin at doses from 1 to 20.5 mg/day for 42 days [6, 7].

### Toxicological studies

The Committee previously concluded that the NOAEL for lutein was 200 mg/kg bw per day, the highest dose tested in a 13-week rat toxicity study, and 1000 mg/kg bw per day, the highest dose tested in a rat developmental toxicity study. For lutein esters from *Tagetes erecta*, the NOAEL was 1000 mg/kg bw per day (equivalent to 540 mg/kg bw per day of lutein), the highest dose tested in both a 13-week and a developmental toxicity study in rats.

The studies reviewed by the Committee confirm that lutein, zeaxanthin and *meso*-zeaxanthin, tested in their free form, are of very low toxicity. The only substance-related finding was discoloration of faeces and fur or skin in animals at higher doses. The NOAELs in short-term and long-term toxicity studies were approximately 210–400 mg/kg bw per day for lutein, 87.5–260 mg/kg bw per day for zeaxanthin and 200–300 mg/kg bw per day for *meso*-zeaxanthin in rats and 10 mg/kg bw per day for lutein and zeaxanthin in monkeys. These NOAELs were generally the highest dose levels tested [8, 9, 10].

A 2-generation reproductive toxicity study in rats found that administration of zeaxanthin at up to 500 mg/kg bw per day, the highest dose tested, did not cause any adverse effects [11].

Based on previously and newly available data, the Committee considered that lutein, zeaxanthin and *meso*-zeaxanthin did not raise concerns with respect to genotoxicity.

## Observations in humans

The Committee previously reviewed the results of clinical studies in which lutein and lutein esters from *Tagetes erecta* were given as nutritional supplements or therapeutic agents for age-related macular degeneration ([Annex 1](#), references 173 and 220). Although these studies were not designed as safety assessments, lutein and lutein esters were found to be well tolerated. This was confirmed based on evaluations of newly available clinical studies. These clinical studies included healthy and preterm infants given lutein in infant formula at concentrations equal to 0.5 mg/kg bw per day for 6 weeks. Doses for adults were up to 10 mg/person per day [4, 12].

## Assessment of dietary exposure

At the sixty-third meeting, the Committee estimated mean and 90th percentile dietary exposure to lutein from *Tagetes erecta* as approximately 7 and 13 mg/day, respectively (equivalent to 0.12 and 0.22 mg/kg bw per day, assuming a 60 kg body weight) ([Annex 1](#), reference 173).

At the seventy-ninth meeting, the use of lutein esters from *Tagetes erecta* was considered to be substitutional for the use of lutein from *Tagetes erecta*.

The present Committee estimated exposure to lutein, zeaxanthin and their esters from *Tagetes erecta* and zeaxanthin (synthetic). When used as a food colour, these substances are substitutional on a molar basis, and exposures are expressed as lutein throughout the report. The estimates included exposure from the use in food supplements. Exposure to *meso*-zeaxanthin was not explicitly assessed by the Committee although it is present in some commercial food supplements.

A comprehensive literature search retrieved 20 additional references relevant to the dietary exposure assessment.

Dietary exposure from the use of lutein, zeaxanthin and their esters and zeaxanthin (synthetic) as a food colour was estimated as 0.3 mg/kg bw per day for adults and 1 mg/kg bw per day for children by EFSA [13]. These estimates, based on maximum reported use levels and national consumption data from 11 European countries, were higher than those estimated by the Committee at the sixty-third meeting.

Dietary exposure from the use of lutein, zeaxanthin and their esters and zeaxanthin (synthetic) in food supplements was estimated by the Committee from dosage information on product labels and from intervention studies to be 0.67 mg/kg bw per day for both adults and children.

Dietary exposure from natural occurrence in food was estimated as 0.13 mg/kg bw per day for adults and 0.69 mg/kg bw per day for children. These estimates were based on national food consumption data from China for adults

Table 7

**Estimates of high dietary exposure to lutein, zeaxanthin and their esters from *Tagetes erecta* and zeaxanthin (synthetic)**

Source of exposure	Estimated dietary exposure, mg/kg bw per day (%) <sup>a</sup>	
	Adults	Children
Food colour	0.4 (33)	1 (42)
Food supplements	0.67 (56)	0.67 (28)
Natural occurrence	0.13 (11)	0.69 (29)
Aggregated exposure	1.2	2.4

bw: body weight

<sup>a</sup> Estimated dietary exposure in mg/kg bw per day and, in parentheses, the estimated exposure as a percentage of the total (aggregated) exposure.

[14] and from the Republic of Korea for children [15]. These estimates were also higher than the previous Committee's estimates.

The present Committee estimated a conservative aggregated high dietary exposure from the use of lutein, zeaxanthin and their esters from *Tagetes erecta* and zeaxanthin (synthetic) as food colour and from food supplements in combination with the natural occurrence of these xanthophylls to be 1.2 mg/kg bw per day for adults and 2.4 mg/kg bw per day for children (Table 7).

## Evaluation

Free lutein, lutein esters and free zeaxanthin including *meso*-zeaxanthin are biochemically and toxicologically equivalent. The esters of lutein and zeaxanthin are hydrolysed in the gastrointestinal tract, and systemic exposure is to free lutein and zeaxanthin, compounds that differ only in the position of a single double bond. Lutein and zeaxanthin are naturally present in food. In addition, both xanthophylls are essential constituents of the primate retina; biologically controlled mechanisms and pathways exist for absorption of both xanthophylls from the diet and their distribution within the body and deposition in the retina.

The Committee concluded that there were sufficient toxicological data to complete a safety assessment of lutein and lutein esters from *Tagetes erecta*, synthetic zeaxanthin and *meso*-zeaxanthin. The Committee considered the available toxicological data together with the dietary exposure of the general population.

No adverse effects were observed in a broad range of toxicological studies of free lutein, lutein esters and free zeaxanthin and *meso*-zeaxanthin in laboratory animals and in clinical studies in humans. Results from a new 2-generation reproductive toxicity study of zeaxanthin in rats indicated no adverse effects at up to 500 mg/kg bw per day, the highest dose tested [11].



The estimated dietary exposure from the use of free lutein, lutein esters and free zeaxanthin as colours or food supplements are in the same order of magnitude as the intakes from foods where these xanthophylls are naturally present.

Based on the absence of toxicity in a wide range of studies with NOAELs of approximately 210–400 mg/kg bw per day for lutein, 87.5–500 mg/kg bw per day for zeaxanthin and 200–300 mg/kg bw per day for *meso*-zeaxanthin and estimated dietary exposure of up to 2.4 mg/kg bw per day, the Committee established a group ADI “not specified” for lutein from *Tagetes erecta*, lutein esters from *Tagetes erecta* and zeaxanthin (synthetic).

*Meso*-zeaxanthin was not included in this group ADI as specifications are not currently available.

The group ADI of 0–2 mg/kg bw for lutein from *Tagetes erecta* and zeaxanthin (synthetic) was withdrawn.

A toxicological and dietary exposure monograph addendum was prepared.

The specifications for lutein from *Tagetes erecta* were revised, and the Chemical and Technical Assessment was updated. The specifications for lutein esters from *Tagetes erecta* and zeaxanthin (synthetic) were maintained.

## References

1. Nolan JM, Meagher K, Kashani S, Beatty S. What is *meso*-zeaxanthin, and where does it come from? *Eye* (Lond). 2013;27(8):899–905.
2. Sheshappa MB, Ranganathan A, Bhatiwada N, Talahalli RR, Vallikannan B. Dietary components affect the plasma and tissue levels of lutein in aged rats with lutein deficiency—a repeated gavage and dietary study. *J Food Sci*. 2015;80(10):2322–30.
3. Evans M, Beck M, Elliott J, Etheve S, Roberts R, Schalch W. Effects of formulation on the bioavailability of lutein and zeaxanthin. A randomized, double-blind, cross-over, comparative, single-dose study in healthy subjects. *Eur J Nutr*. 2013;52(4):1381–91.
4. Khachik F, de Moura FF, Chew EY, Douglass LW, Ferris FL, Kim J et al. The effect of lutein and zeaxanthin supplementation on metabolites of these carotenoids in the serum of persons aged 60 or older. *Invest Ophthalmol Vis Sci*. 2006;47(12):5234–42.
5. Albert GI, Hoeller U, Schierle J, Neuringer M, Johnson EJ, Schalch W. Metabolism of lutein and zeaxanthin in rhesus monkeys. Identification of (3R,6'R)- and (3R,6'S)-3'-dehydro-lutein as common metabolites and comparison to humans. *Comp Biochem Physiol B Biochem Mol Biol*. 2008;151(1):70–8.
6. Hartmann D, Thürmann PA, Spitzer V, Schalch W, Manner B, Cohn W. Plasma kinetics of zeaxanthin and 3'-dehydro-lutein after multiple oral doses of synthetic zeaxanthin. *Am J Clin Nutr*. 2004;79(3):410–7.
7. Thürmann PA, Schalch W, Aebischer JC, Tenter U, Cohn W. Plasma kinetics of lutein, zeaxanthin and 3'-dehydro-lutein after multiple oral doses of a lutein supplement. *Am J Clin Nutr*. 2005;82(1):88–97.
8. Ravikrishnan R, Rusia S, Ilamurugan G, Salunkhe U, Deshpande J, Shankaranarayanan ML et al. Safety assessment of lutein and zeaxanthin (Lutemax 2020): subchronic toxicity and mutagenicity studies. *Food Chem Toxicol*. 2011;49(11):2841–8.

9. Thurnham DI, Howard AN. Studies on *meso*-zeaxanthin for potential toxicity and mutagenicity. *Food Chem Toxicol.* 2013;59:455–63.
10. Ravi KB, Raghunatha Reddy KR, Shankaranarayanan J, Deshpande JV, Juturu V, Soni MG. Safety evaluation of zeaxanthin concentrate (OmniXan™): acute, subchronic toxicity and mutagenicity studies. *Food Chem Toxicol.* 2014;72:30–9.
11. Clode S. Zeaxanthin 10% WS beadlets (Ro 01-9509): Two generation oral (dietary administration) reproduction toxicity study in the rat. Amendment to final report study no.: 2416/001. Unpublished study by Covance Laboratories Ltd, Harrogate, North Yorkshire, England, United Kingdom; 2018. Submitted to WHO by DSM Nutritional Products Ltd, Kaiseraugst, Switzerland.
12. Costa S, Giannantonio C, Romagnoli C, Vento G, Gervasoni J, Persichilli S et al. Effects of lutein supplementation on biological antioxidant status in preterm infants: a randomized clinical trial. *J Matern Fetal Neonatal Med.* 2013;26(13):1311–5.
13. Statement on the safety assessment of the exposure to lutein preparations based on new data on the use levels of lutein. *EFSA J.* 2012;10(3):2589. doi:10.2903/j.efsa.2012.2589.
14. Wang L, Li B, Pan MX, Mo XF, Chen YM, Zhang CX. Specific carotenoid intake is inversely associated with the risk of breast cancer among Chinese women. *Br J Nutr.* 2014;111(9):1686–95.
15. Lee HS, Cho YH, Park J, Shin HR, Sung MK. Dietary intake of phytonutrients in relation to fruit and vegetable consumption in Korea. *J Acad Nutr Diet.* 2013;113(9):1194–99.

### 3.1.6 Neutral methacrylate copolymer

#### Explanation

Neutral methacrylate copolymer (NMC; E 1206; INS No. 1206; CAS No. 9010-88-2; ethyl acrylate methyl methacrylate polymer; ethyl acrylate methyl methacrylate polymer; ethyl acrylate polymer with methyl methacrylate; methyl methacrylate ethyl acrylate polymer; methyl methacrylate polymer with ethyl acrylate) is a copolymer manufactured from the monomers ethyl acrylate and methyl methacrylate in the molar ratio of 2:1. NMC is described in the European Pharmacopoeia [1] and United States Pharmacopoeia and National Formulary [2].

The Committee considered three different copolymers: basic, anionic and neutral methacrylate copolymers. Each copolymer releases the active ingredients from within their coatings under different physiological conditions in different parts of the digestive tract. NMC is insoluble in aqueous media and its release is not pH dependent. NMC is used in sustained release formulations that enable continuous dissolution of an active ingredient over a defined time. Release periods can be controlled by changing the amount of copolymer used.

NMC has not previously been considered by the Committee. The Committee evaluated the use of NMC as a coating or glazing agent for solid food supplements such as capsules, pastilles, tablets, pills, pellets and powders, at levels not exceeding 20%, at the request of CCFA49 [3]. NMC is also used in pharmaceuticals.

Toxicological data submitted for evaluation included absorption, distribution and excretion studies, acute and short-term toxicity and genotoxicity studies as well as a developmental toxicity study. Limited data were also submitted on the residual monomers. A comprehensive literature search retrieved data on the residual monomers but no other studies on NMC. The Committee also considered the data on the residual monomers because the monomers are of low molecular weight and therefore likely to be absorbed from the gastrointestinal tract. Due to the low levels of residual monomers present in NMC, the Committee considered only the ADME, long-term toxicity and genotoxicity data on the monomers.

The Committee was also aware that NMC contains an oligomer fraction of between 0.06% and 0.13%. As the lower end of the molecular weight range for all the constituent oligomers is greater than 5000 Da, it is unlikely that they would be absorbed from the gastrointestinal tract. Therefore, the Committee did not consider the toxicological aspects of the oligomers.

Unless otherwise stated, the test substance used in the absorption, distribution and excretion and toxicity studies was prepared from an aqueous dispersion with 0.7% emulsifier and then freeze-dried to remove water. In all cases, doses have been expressed as dry weight of NMC.

The Committee evaluated toxicological and exposure data on polyethylene glycol monostearyl ether, methanol and ethanol, residual components of NMC that can be present in the final product because they are used in the manufacture of the copolymer. The Committee concluded that these residual components do not pose a safety concern at the maximum estimated exposure levels.

### **Chemical and technical considerations**

NMC is manufactured by emulsion polymerization of the monomers ethyl acrylate and methyl methacrylate with water-soluble radical initiators. The product is purified by water vapour distillation and filtration to remove residual monomers, excess water, other volatile low molecular weight substances and coagulum.

NMC has a weight-average molecular weight of 600 000 Da and a number-average molecular weight of 220 000 Da.

Although organic solvents are not used in the manufacture of NMC, methanol may be present at a level not exceeding 100 mg/kg and ethanol may be present at a level not exceeding 1000 mg/kg. The copolymer is standardized as a 30% aqueous dispersion with polyethylene glycol monostearyl ether (0.7%). The copolymer dispersion may contain the residual monomers methyl methacrylate (not more than 50 mg/kg) and ethyl acrylate (not more than 20 mg/kg).

## Biochemical aspects

### NMC

In a pre-GLP study, NMC was found to be poorly absorbed and quickly eliminated from the body when single doses of  $^{14}\text{C}$ -labelled NMC at 600 mg/kg bw per day were administered to rats by gavage. An average of 97.6% of the radioactivity was excreted in the faeces within 48 hours. Seven days after dosing, levels of radioactivity in tissues of treated animals did not differ significantly from that of control animals [4].

### Residual monomers

Methyl methacrylate is rapidly absorbed and distributed following inhalation or oral administration to rats. Methyl methacrylate is metabolized to methacrylic acid and methanol, which is subsequently converted to carbon dioxide via the tricarboxylic acid cycle in both experimental animals and humans [5, 6, 7].

Ethyl acrylate is rapidly absorbed and metabolized following inhalation or oral administration in rats. Metabolism occurs via hydrolysis of the ester linkage by carboxylesterases, forming ethanol and acrylic acid, both of which are ultimately metabolized to carbon dioxide, or via conjugation of ethyl acrylate with glutathione. Following conjugation with glutathione, ethyl acrylate is rapidly eliminated via urinary excretion [8].

## Toxicological studies

### NMC

Two acute toxicity studies were available, in rats and in dogs. Rats received doses of 25.2–28.2 g/kg bw in their feed; dogs received doses of 7950 or 9100 mg/kg bw in their feed. No treatment-related effects were seen during the observation period or at macroscopic examination of the organs. Urine analysis also found no treatment-related effects [9, 10].

Two short-term toxicity studies were available in rats. In the first study, doses of 0, 500, 1000 or 2000 mg/kg bw per day were given by gavage for 35 days. It is not clear if the doses were expressed in dry weight of polymer or of the preparation. No treatment-related effects were observed [11]. In the second study, doses of 0, 500 or 2000 mg/kg bw per day were given in the feed for 6 months. No treatment-related effects were observed [12]. In both studies, the NOAELs were 2000 mg/kg bw per day, the highest doses tested. In a 26-week study, dogs were given NMC at doses of 0, 50, 125 or 250 mg/kg bw per day administered as NMC-coated cellulose pellets. Apart from decreases in body-weight gain and feed consumption at the highest dose, and the presence of white granular material in the gut, attributed to the physical characteristics of the test material, no treatment-related effects were observed. The NOAEL was 250 mg/kg

bw per day, the highest doses tested [13]. A 28-day study in mini pigs, in which NMC was given by gavage as NMC-coated cellulose pellets, the calculated doses were 0, 113, 227 and 454 mg/kg bw per day. Other than the presence of white granular material in the intestines, attributed to the physical characteristics of the test substance, no treatment-related effects were found. The NOAEL was 454 mg/kg bw per day, the highest dose tested [14].

Two reverse mutation studies in bacteria, one in vitro mouse lymphoma assay and an in vivo mouse micronucleus assay gave negative results. The Committee concluded that the NMC does not give rise to concern for genotoxicity.

No long-term toxicity or carcinogenicity studies with NMC were available.

Developmental toxicity studies were available in rats and rabbits, both using doses of 0, 500 or 2000 mg/kg bw per day in the feed during the period of organogenesis. No treatment-related effects were observed. The NOAEL for both studies was 2000 mg/kg bw per day, the highest doses tested.

Studies on cytotoxicity and dermal, inhalation and ocular toxicity found no effects.

## Residual monomers

### *Methyl methacrylate*

In a long-term toxicity study in rats given methyl methacrylate in drinking-water at 0, 6, 60 or 2000 mg/L (equal to 0, 0.4, 4 and 121 mg/kg bw per day for males and 0, 0.5, 5 and 146 mg/kg bw per day for females, respectively) for 2 years, relative kidney weight increased in females at the highest dose but no treatment-related histopathological effects were observed in any organs or tissues [15]. Based on the results of this study, a TDI of 1.2 mg/kg bw per day was determined [16].

In long-term toxicity and carcinogenicity studies in mice, rats and hamsters given methyl methacrylate by inhalation, the observed effects were, in general, similar to those reported in the short-term toxicity studies but also included inflammation and epithelial hyperplasia of the nasal cavity and degeneration of the olfactory sensory epithelium. There was no evidence of any carcinogenic effects [15].

Bacterial reverse mutation assays with methyl methacrylate gave mostly negative results. Mixed results (i.e. positive, weakly positive or negative) were obtained in in vitro chromosomal aberration and SCE assays. One in vitro micronucleus assay was unequivocally negative, whereas a second assay was negative at low concentrations but weakly positive at higher concentrations. Three mouse lymphoma assays for gene mutations were positive. A mouse bone marrow micronucleus assay was negative, but it is not clear if the target tissue was exposed to the test substance. A rat micronucleus assay with exposure by inhalation was positive following 1 day of exposure but negative following 5 days

of exposure. These results were judged by the Committee to be inconclusive. The Committee concluded that there was some evidence of mutagenicity and clastogenicity *in vitro*. There was a lack of adequate *in vivo* tests following up the equivocal findings.

### *Ethyl acrylate*

Studies in mice and rats administered ethyl acrylate at 0, 100 or 200 mg/kg bw per day for 103 weeks by gavage showed an increased incidence of squamous cell hyperplasia and papillomas in the forestomach of both species, and, in male rats, squamous cell carcinomas in the forestomach. In contrast, studies using other routes of administration including via inhalation (with concentrations up to 310 mg/m<sup>3</sup>) and in the drinking-water (with doses up to 280 mg/kg bw per day) showed no such effects. An IARC working group concluded that the mechanism of formation of the forestomach tumours in rodents is not relevant to humans; rather it can be attributed to the irritating effect of high bolus doses of ethyl acrylate delivered to the contact site (forestomach) by gavage [17]. This conclusion was recently confirmed by Health Canada [8]. The Committee concluded that ethyl acrylate is not a carcinogenic risk to humans.

Genotoxicity results for ethyl acrylate are mixed, with some positive results *in vitro*, some negative results in reverse mutation assays in bacteria and positive results in a mouse lymphoma assay. There were both positive and negative results in *in vitro* SCE assays and in chromosomal aberration assays with metabolic activation, but no evidence of clastogenicity in the absence of metabolic activation.

In *in vivo* studies on ethyl acrylate, two early mouse micronucleus studies using intraperitoneal dosing gave positive results, although one of these gave positive results in only one of the five experiments carried out [18, 19]. However, three other micronucleus studies in mice (one dermal exposure, two by intraperitoneal injection) all gave negative results ([Annex 1](#), reference 174). A chromosomal aberration assay and an *in vivo* SCE assay and a recent point mutation assay in mice were also negative. The Committee concluded that the genotoxic potential observed in some *in vitro* studies was not expressed *in vivo*. The Committee noted that Health Canada [8] reached a similar conclusion.

### **Observations in humans**

No human data were available on NMC.

### **Assessment of dietary exposure**

The Committee evaluated exposure to NMC from its use as a glazing or coating agent in food supplements and foods for special medical purposes. As another

major use of NMC is in pharmaceuticals, this use was also considered in the exposure assessment. The level of use of NMC is a maximum of 20%.

The Committee evaluated exposure to NMC for the copolymer and its monomers, methyl methacrylate and ethyl acrylate. The exposure assessment included estimates submitted by the sponsor and an evaluation by EFSA [20] based on consumption of food supplements and pharmaceuticals. The Committee also estimated exposure based on national food consumption data for food supplements using the concentration proposed by the sponsor. The national consumption data were from CIFOCOs and data submitted to the Committee from Australia and New Zealand. A comprehensive literature search was also conducted; no additional studies relevant to the exposure assessment were found.

No quantitative estimates of exposure could be determined for foods for special medical purposes. The sponsor indicated that it is not anticipated that foods for special medical purposes would increase exposures above that of food supplements and pharmaceuticals given the conservative nature of those calculations. In addition, the consumers of foods for special medical purposes will generally be under medical supervision, and exposures for these consumers are therefore not relevant for the general healthy population. This use was therefore not further considered by the Committee.

The total monomeric content of NMC is less than 0.01%. This level was used to calculate the exposure to total monomers from the copolymer exposure. Estimates of exposure to the individual monomers were based on the exposure to total monomers, taking into account the ratio of each individual monomer in the copolymer.

All estimates of exposure are presented as a range from the lowest of the average exposures to the highest of the high exposures.

The estimated exposures to NMC and its monomers from uses in food supplements are shown in [Table 8](#).

The Committee noted that NMC is used in pharmaceuticals. Estimated exposures from this use from the sponsor and EFSA [20] ranged between 10.0 and 23.3 mg/kg bw per day for adults and children. These estimates were within the range of exposures from food supplements. However, the Committee considered that such use should not be taken into account in the assessment of long-term dietary exposure for a healthy population.

## Evaluation

New specifications for NMC were prepared and made tentative, requiring a suitable validated method for its assay. A Chemical and Technical Assessment was prepared.

The Committee concluded that the use of NMC that complies with the specifications established at the current meeting is not of safety concern when the

Table 8

**Summary of range of estimated exposures to NMC and its monomers from uses in food supplements for average and high exposures**

Population group	Range of estimated dietary exposures <sup>a,b</sup>		
	Copolymer exposure (mg/kg bw per day)	Monomer exposure (µg/kg bw per day)	
		Methyl methacrylate	Ethyl acrylate
Adults	6.9–74	0.2–2.2	0.5–5.2
Children	5.8–86	0.2–2.5	0.4–6.0

bw: body weight; NMC: neutral methacrylate copolymer

<sup>a</sup> All estimates of exposure are presented as a range from the lowest of the average exposures to the highest of the high exposures. The lower end of each range is the lowest of the estimated mean exposures, and the upper end of each range is the highest of the estimated high exposures.

<sup>b</sup> Includes exposure estimates submitted by the sponsor and EFSA (2010) and national estimates calculated by the Committee, based on a concentration of 200 mg per 1 g dosage unit.

food additive is used as a coating or glazing agent for solid food supplements and for foods for special medical purposes.

The Committee therefore established an ADI “not specified” for NMC. The ADI “not specified” was made temporary because the specifications are tentative.

The available toxicology data for NMC do not give rise to concerns for toxicity. The substance is poorly absorbed and is excreted in the faeces. In short-term and developmental toxicity studies, the NOAELs for NMC range from 454 to 2000 mg/kg bw per day, and these were the highest doses tested. Estimated exposures to NMC range from 5.8 to 86 mg/kg bw per day.

Toxicological data on the residual monomers do not give rise to concerns when taking into account the low exposures. Genotoxicity data for methyl methacrylate suggest a potential risk for mutagenicity and clastogenicity *in vitro*, and there is a lack of adequate data on genotoxicity *in vivo*. However, in carcinogenicity studies in mice, rats and hamsters given methyl methacrylate by inhalation, there was no evidence of any carcinogenic effects. In a 2-year drinking-water study on methyl methacrylate in rats, the NOAEL was 121 mg/kg bw per day, from which a TDI of 1.2 mg/kg bw per day was derived [16]. Estimated exposures to methyl methacrylate range from 0.2 to 2.5 µg/kg bw per day, which are below the TDI.

Although there were some positive genotoxicity findings for ethyl acrylate, the Committee concluded that the genotoxic potential observed in some *in vitro* studies was not expressed *in vivo*. Long-term toxicity studies on ethyl acrylate in mice and rats produced forestomach tumours, but the Committee concurred with the conclusions of IARC [17] and, more recently, Health Canada [8], that the mechanism of forestomach tumour formation in rats and mice is not relevant to humans. The Committee was also reassured by the long-term carcinogenicity studies on ethyl acrylate that did not use gavage as the route of



administration; these suggest that ethyl acrylate is not carcinogenic at the doses tested. In the 2-year drinking-water study on ethyl acrylate, the NOAEL was 280 mg/kg bw per day. Estimated dietary exposures to ethyl acrylate range from 0.4 to 6 µg/kg bw per day. The margin of exposure (MOE) based on the highest estimated exposure was calculated to be 46 000.

Assessments of dietary exposure to methyl methacrylate and ethyl acrylate due to their residual occurrence in NMC suggest that exposure to these monomers from the uses of NMC is not a safety concern.

A toxicological and dietary exposure monograph was prepared.

## References

1. Polyacrylate Dispersion 30 Per Cent. 9 01/2017:0733. European Pharmacopeia; 2017.
2. United States Pharmacopeia and National Formulary (USP 41-NF 36); Ethyl acrylate and methyl methacrylate copolymer dispersion. Vol. 1. Rockville, Maryland: United States Pharmacopeial Convention; 2017.
3. 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 and Geneva: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2017 (REP17/FA).
4. The excretion and tissue distribution of radioactivity after oral administration to rats. Huntingdon Life Sciences, Suffolk, England, United Kingdom; 1986. Report no. RTS 010/984481(2nd amendment). Unpublished study report submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
5. Bratt H, Hathway DE. Fate of methyl methacrylate in rats. *Br J Cancer*. 1977;36(1):114–9 [cited in reference 16].
6. Canadian Environmental Protection Act: Priority Substances List assessment report: Methyl methacrylate. Prepared by Health Canada and Environment Canada. Ottawa: Government of Canada; 1993 (Cat. No.: En40-215/10E) [cited in reference 16].
7. Methyl methacrylate – CAS No. 80-62-6. Brussels: European Centre for Ecotoxicology and Toxicology of Chemicals; 1995. (Joint Assessment of Commodity Chemicals No. 30) [cited in reference 16].
8. Screening assessment for the challenge 2-propenoic acid, ethyl ester (ethyl acrylate). Chemical Abstracts Service Registry Number 140-88-5. Ottawa (ON): Environment Canada, Health Canada; 2011.
9. The acute toxicity of preparations 2807B and 2837B on oral administration in rats. Unpublished study by Laboratory for Pharmacology and Toxicology, Hamburg-Hausbruch, Germany; 1971. Submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
10. The acute toxicity of preparations 2807B and 2837B on oral administration in dogs when administered in the feedstuff. Unpublished study by Laboratory for Pharmacology and Toxicology, Hamburg-Hausbruch, Germany; 1972a. Submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
11. Subacute toxicity study with rats by oral administration for one month and the recovery study. Unpublished study by BoZo Research Centre, Co Ltd, Japan; 1981. Submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.

12. Six-month toxicity of 2837D in Sprague Dawley rats by administration in the diet. Unpublished study report by Laboratory for Pharmacology and Toxicology, Hamburg-Hausbruch, Germany; 1972b. Submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
13. 26-Week oral toxicity study in dogs followed by a 3-week recovery period. Unpublished study report by Research Toxicology Centre, Rome, Italy; 2007a. Submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
14. 4-Week oral toxicity study in minipigs. Unpublished study report no. 31030 by Research Toxicology Centre, Rome, Italy; 2006. Submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
15. Borzelleca JF, Larson PS, Hennigar GR Jr, Huf EG, Crawford EM et al. Studies on the chronic oral toxicity of monomeric ethyl acrylate and methyl methacrylate. *Toxicol Appl Pharmacol.* 1964;6:29–36 [cited in reference 16].
16. Methyl methacrylate. Geneva: World Health Organization; 1998. (International Programme on Chemical Safety II. Concise International Chemical Assessment Document No. 4).
17. Predictive value of rodent forestomach and gastric neuroendocrine tumours in evaluation carcinogenic risks to humans. Views and Expert Opinions of an IARC Working Group. Lyon: International Agency for Research on Cancer, World Health Organization; 2003 (IARC Technical Publication No. 39) [cited in [Annex 1](#), reference 174].
18. Przybojewska B, Dziubałtowska E, Kowalski Z. Genotoxic effects of ethyl acrylate and methyl acrylate in the mouse evaluated by the micronucleus test. *Mutat Res.* 1984;135(3):189–91.
19. Ashby J, Richardson CR, Tinwell H. Inactivity of ethyl acrylate in the mouse bone marrow micronucleus assay. *Mutagenesis.* 1989;4(4):283–5.
20. Scientific Opinion on the safety of anionic methacrylate copolymer for the proposed uses as a food additive. *EFSA J.* 2010;8(7):1655. doi: 10.2903/j.efsa.2010.1656.

### 3.1.7 Sorbitol syrup

#### Explanation

Sorbitol syrup (INS No. 420(ii)) is currently included in the Codex GSFA ([Annex 3](#)) although it has not been assigned an ADI or determined, on the basis of other criteria, to be safe. At its Fiftieth Session, the Codex Committee on Contaminants in Food Additives [1] requested the Committee to consider the previous evaluations of sorbitol, hydrogenated glucose syrups and other relevant substances and advise on the need for a separate evaluation of sorbitol syrup or if the ADI “not specified” for sorbitol is also applicable for sorbitol syrup.

#### Evaluation

The Committee acknowledges that sorbitol and sorbitol syrup are chemically similar but distinct substances that should be assigned separate ADIs.

Sorbitol syrup consists of not less than 99.0% of hydrogenated saccharides and not less than 50.0% of D-sorbitol on an anhydrous basis. The D-sorbitol portion of sorbitol syrup is not of toxicological concern, and the Committee

established an ADI “not specified” for sorbitol at its twenty-sixth meeting ([Annex 1](#), reference 61).

The remaining portion of sorbitol syrup consists of other hydrogenated saccharides. These hydrogenated saccharides were evaluated previously by the Committee. Evaluations of maltitol syrup ([Annex 1](#), reference 83) and polyglycitol syrup ([Annex 1](#), reference 137) concluded that the presence of maltitol and higher-order polyols in these syrups was not of toxicological concern, and each of the syrups was assigned an ADI “not specified”. The evaluations of these syrups were based on toxicological findings and biochemical aspects.

The hydrogenated saccharides are poorly absorbed. They are partially hydrolysed by enzymes in the gastrointestinal tract and also metabolized by colonic microflora. The previous Committee concluded that these substances were fully metabolized into natural body constituents ([Annex 1](#), reference 70) and not of toxicological concern.

Based on the similarity of the chemical constituents of sorbitol syrup to sorbitol, maltitol syrup and polyglycitol syrup, the Committee concluded that there is no need for a separate evaluation of sorbitol syrup and established an ADI “not specified” for sorbitol syrup.

No toxicological monograph was prepared.

## Reference

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

### 3.1.8 Spirulina extract

#### Explanation

Spirulina extract (INS No. 134) is used as a food additive, as a blue colouring agent. Spirulina extract is used in food in China, the European Union, Japan, Mexico and the USA, among others. It is used as a colour in a wide range of foods and beverages including flavoured dairy products, cheese, dairy-based desserts, processed fruits and vegetables, baked goods and baking mixes, alcoholic and non-alcoholic beverages and beverage bases, breakfast cereals, cocoa products, confectionery products (including soft and hard candy and chewing gum), egg products, gravies and sauces, herbs and spices, condiments and soup and soup mixes. It is also used as a colouring agent in nutritional supplements and in pharmaceuticals. Intended use levels range from 400 to 40 000 mg/kg, depending on the food item or category and the colour strength of the formulated spirulina extract used.

Spirulina extract has not been evaluated previously by the Committee. It was placed on the agenda for its use as a food colour at the request of CCFA49 [1].

A toxicological dossier submitted by the sponsor summarized the available toxicity data on spirulina, together with relevant study reports and publications. A few of the studies were conducted using spirulina extract, but most toxicity studies used dried spirulina as the test material. A comprehensive literature search retrieved an additional 17 relevant toxicological study reports.

### Chemical and technical considerations

Spirulina extract is produced from *Arthrospira platensis* (commonly called *Spirulina platensis*), an edible cyanobacterium cultivated in open or covered ponds or in bioreactors. Commercial cultivation occurs in alkaline aqueous medium containing sodium bicarbonate, nitrates, phosphates, sulfates and other nutrients including trace minerals. No herbicides or additional solvents are used during the cultivation of *A. platensis* or in the manufacture of spirulina extract. Cultivation conditions are optimized to control for contaminating organisms.

Following cultivation, the culture is harvested, concentrated, washed and prepared as a paste or dried to a powder. The dried or fresh biomass undergoes aqueous extraction and pH adjustment, as needed. Following extraction, the mixture undergoes centrifugation and filtration to remove cell debris and water-insoluble components. The resulting aqueous phase contains proteins, carbohydrates, minerals and two phycobiliproteins (also referred to as phycocyanins) that impart the blue colour. The mixture is concentrated to the desired pigment concentration and then pasteurized and/or sterilized before packaging. Spirulina extract products may undergo additional standardization and/or drying to achieve the desired formulation and pigment concentration. Commercially available products occur in either liquid (aqueous) or powder form with a wide range of pigment concentrations.

The primary colouring principles in spirulina extract are C-phycocyanin (CAS No. 11016-15-2; EINECS No. 234-248-8; ~30 kDa) and allophycocyanin (no CAS number assigned; ~105 kDa), in various ratios, with C-phycocyanin occurring in higher proportions. Phycocyanins are complexes of proteins with the pigment molecule phycocyanobilin [2]. The total content of C-phycocyanin and allophycocyanin in spirulina extract varies depending on the desired colour effect and degree of dilution of the extract. Phycocyanin concentrations in spirulina extract range from 1.5–15% in liquid products to 1.5–65% in powder products, as the sum of C-phycocyanin and allophycocyanin.

Commercial spirulina extracts typically contain peptides and proteins (10–90%, dry weight, including the proteins complexed with phycocyanobilin), carbohydrates and polysaccharides (≤65%, dry weight), fat (<1%, dry weight), fibre (<6%, dry weight), minerals/ash (<6%, dry weight) and water (<6% for

powder products and  $\leq 95\%$  for liquid formulations). Spirulina extract may contain trace amounts ( $< 1\%$ ) of carotenoids and chlorophylls, which are largely removed during production.

### Biochemical aspects

ADME data on spirulina extract are not available.

Spirulina extract is described as consisting of various proportions of proteins and carbohydrates as well as much smaller amounts of fibre and fats. These extract components are digested through normal biochemical pathways like other common dietary constituents.

An in vitro simulated gastric fluid digestion assay demonstrated that the protein portion of the C-phycoerythrin is rapidly digested by pepsin into small chromopeptides, consisting of two to 13 amino acids [3]. Because the chemical structure of the chromophore phycocyanobilin is very similar to biliverdin, a non-reduced form of bilirubin [4], phycocyanobilin is expected to be metabolized and excreted like bilirubin, through the bile and into the faeces [5, 6].

No evidence of bioaccumulation of coloured matter was observed in repeated-dose toxicity studies in laboratory animals fed spirulina extract or dried spirulina.

### Toxicological studies

As noted, most of the tests conducted used dried spirulina and not spirulina extract. However, based on the similarity of the constituents and the high concentrations of the dried spirulina test materials used in the toxicity studies, the Committee considered this acceptable in the evaluation of spirulina extract. In some studies conducted with spirulina extract, the phycocyanin content was reported; no study conducted with dried spirulina reported its phycocyanin content. The Committee also noted that the source of the test material in some studies was *Spirulina maxima*, as opposed to *S. platensis*. Given the chemical, genetic and nutritional similarity of these two species of edible cyanobacteria [7, 8, 9], this was considered acceptable in the evaluation of spirulina extract. Most reports did not state if the studies were GLP or guideline compliant. However, the Committee concluded that the studies were of acceptable quality and the findings valid.

In acute gavage toxicity studies, no clinical signs of toxicity were observed in mice administered spirulina extract (phycocyanin content not reported) at a dose of up to 3000 mg/kg bw [10]; in rats administered spirulina extract (24–26% phycocyanin content) at a dose of up to 5000 mg/kg bw [11, 12]; or in rats administered dried spirulina at a dose of up to 10 000 mg/kg bw [13].

No toxicity was seen in rats administered spirulina extract (24% phycocyanin content) by gavage at a dose of 3000 mg/kg bw per day for 14 days

[12]; in rats administered spirulina extract (phycocyanin content not reported) by gavage at doses of up to 4000 mg/kg bw per day for 12 weeks [14]; in rats fed diets with spirulina extract (26% phycocyanin content) at a concentration of 0.4% (equivalent to 400 mg/kg bw per day) for 14 weeks [11]; or in rats in a long-term toxicity study where the dietary concentration of spirulina extract (8–9% phycocyanin content) was 1.0% (equivalent to 500 mg/kg bw per day) for 12 months [15].

In a short-term mouse toxicity study conducted with dietary concentrations of dried spirulina of up to 5% (equivalent to 7500 mg/kg bw per day) for 6 months, the NOAEL was the highest concentration tested [16]. In a set of well-conducted toxicity studies conducted with dietary concentrations of dried spirulina of up to 30% for 13 weeks, the NOAELs were the highest concentrations tested (equivalent to 45 000 and 30 000 mg/kg bw per day in mice and rats, respectively) [17, 18]. Several additional short-term feeding studies conducted with dried spirulina in mice [19] and rats [13, 20, 21] showed no toxicity under the conditions of the studies. Similarly, no evidence of systemic toxicity or carcinogenicity was observed in long-term feeding studies in rats where the dietary concentration of dried spirulina was 30% (equivalent to 15 000 mg/kg bw per day) for 84 weeks [22].

With dried spirulina, a dose-related increase in relative seminal vesicle weight was the only treatment-related effect observed in 13-week-long toxicity studies in mice [18] and rats [17]. This finding was not reproduced in other short-term or long-term toxicity studies where seminal vesicle weight was investigated. As there were no histopathological changes in the seminal vesicles and no effects on reproduction in the test animals, the Committee considered this finding of no toxicological relevance.

A bacterial reverse mutation assay and *in vitro* and *in vivo* chromosomal aberration assays with spirulina extract (26% phycocyanin content) showed no evidence of genotoxicity [12]. Dominant lethal assays conducted in mice and rats with dried spirulina also showed no evidence of genotoxicity under the conditions of the assays [23, 24].

There was no evidence of reproductive toxicity when mice and rats were fed dietary concentrations of dried spirulina of up to 30% (equivalent to 45 000 and 18 000 mg/kg bw per day, respectively) prior to mating (male mice and rats for 9 weeks, female mice for 8 weeks, female rats for 2 weeks) and during mating and gestation. No toxicity was observed in dams or pups when mice, rats and hamsters were fed dietary concentrations of dried spirulina up to 30% (equivalent to 45 000, 30 000 and 27 000 mg/kg bw per day, respectively) over the entire gestation period [25, 26, 27].

In a 2-generation toxicity study in which dosing was limited to F<sub>0</sub> parental females from gestation day 15 through to lactation day 21, mice were fed

diets containing dried spirulina at 0, 10%, 20% or 30% (equivalent to 0, 15 000, 30 000 and 45 000 mg/kg bw per day, respectively). F<sub>1</sub> and F<sub>2</sub> offspring were not directly exposed to the test material. At 30% dried spirulina in the diet, reduced F<sub>1</sub> pup weight at birth and reduced F<sub>1</sub> survival rate on postnatal days 0–4 were reported; no effects were observed in the F<sub>2</sub> animals [26]. The Committee noted that there was no effect on pup survival rate in a 2-generation toxicity study in which F<sub>0</sub> female rats were exposed from gestation day 17 to lactation day 21, in a dosing schedule similar to that used in the Chamorro et al. mouse study [26, 28]. There were also no effects on fetal weight or pup weight at birth in several other reproductive and developmental toxicity studies in the mouse, rat and hamster.

Nutritional studies of dried spirulina fed to rats, rabbits, pigs, sheep and cows showed that the animals maintained good health [29] even when the dietary concentrations were very high (e.g. up to 40% of the diets in rats, equivalent to 40 000 mg/kg bw per day). From a toxicological perspective, these nutritional studies were limited in terms of their observations.

### Observations in humans

Observations in humans included case reports, clinical studies and nutritional studies.

The case reports of adverse effects were relatively few considering the long history and widespread use of dried spirulina as a food ingredient and dietary supplement [30]. A few reports cited adverse immunological reactions, such as allergy, associated with the ingestion of dried spirulina dietary supplements. The Committee noted that dried spirulina was well tolerated in clinical and nutritional studies conducted with gram quantities consumed daily for months.

### Assessment of dietary exposure

Dietary exposure to spirulina extract from its use as a food colour was assessed by the present Committee.

A comprehensive literature search retrieved nine studies relevant for the assessment of dietary exposure. Consumption of spirulina or components of spirulina occurs from uses other than food colour, that is, dried spirulina in dietary supplements, dried spirulina and spirulina extract as food ingredients, and spirulina extract in coatings for dietary supplements and pharmaceuticals. In order to assess the aggregated dietary exposure from these uses, exposures have been normalized based on phycocyanin content. This was considered appropriate since the colour component of spirulina extract is due to its content of phycocyanins (*C*-phycocyanin and allophycocyanin). This approach allowed for a comparison of the two test substances used in the toxicological assessments, dried spirulina and spirulina extract.

Dietary exposure expressed as phycocyanins from the use of spirulina extract as a food colour was estimated using the budget method [31]. The theoretical maximum daily exposure was estimated to be 123 and 385 mg/kg bw per day for adults and children respectively. The conversion to phycocyanin content was based on the content of phycocyanins (28%) in the spirulina extracts proposed for use by the sponsor.

Dietary exposure expressed as phycocyanins from the use of dried spirulina in dietary supplements was estimated to be 33 and 133 mg/kg bw per day for adults and children, respectively. This estimate was based on dosage information on product labels and from intervention studies. The conversion to phycocyanin content was based on phycocyanin content (20%) in products from the high end of the range reported in Generally Recognized as Safe (GRAS) notices for commercial dried spirulina.

Dietary exposure expressed as phycocyanins from the use of dried spirulina or spirulina extract as food ingredients was estimated for high consumers to be 33 and 133 mg/kg bw per day for adults and children, respectively. This estimate was based on proposed uses in four GRAS notices, with the conversion to phycocyanin content based on information in the GRAS documentation.

Dietary exposure expressed as phycocyanins from use of spirulina extract in coatings of dietary supplements was estimated for high consumers to be 0.1 and 0.2 mg/kg per day for adults and children, respectively. The conversion to phycocyanin content was based on the assumption that the concentration was the same as for food colour, that is, 28% in spirulina extract.

The Committee noted that spirulina extract could be used in coatings of pharmaceuticals comparable to the use in coatings for dietary supplements, resulting in a similar dietary exposure (0.1 mg/kg bw per day for adults and 0.2 mg/kg bw per day for children). However, the Committee considered that such use should not be taken into account in the assessment of long-term dietary exposure in a healthy population.

The Committee estimated a conservative aggregated exposure to dried spirulina and spirulina extract from all the assessed uses to be 190 and 650 mg/kg bw per day for adults and children, expressed as phycocyanins (Table 9). Based on this assessment, the estimated exposure to phycocyanins from the use of dried spirulina and spirulina extract as a food colour contributes approximately 60% to this total exposure and as dietary supplements and food ingredients contributes approximately 20% each, while the contribution from the use in coatings of dietary supplements is negligible.

## Evaluation

The Committee established a temporary ADI “not specified” for spirulina extract. The ADI was based on the absence of toxicity in repeated-dose animal studies



Table 9

**Estimates of dietary exposure to phycocyanins from the use of spirulina products**

Use (source of phycocyanins)	Exposure to phycocyanins, mg/kg bw per day (%) <sup>a</sup>	
	Adults	Children
Food colour (spirulina extract)	123 (65)	385 (59)
Dietary supplements (dried spirulina)	33 (17)	133 (20)
Food ingredients (dried spirulina and spirulina extract)	33 (17)	133 (20)
Dietary supplement coating (spirulina extract)	0.1 (0.06)	0.2 (0.03)
Aggregated exposure	190	650

bw: body weight

<sup>a</sup> Estimated dietary exposure in mg/kg bw per day and, in parentheses, the estimated exposure as a percentage of the total (aggregated) exposure.

conducted with spirulina extract and dried spirulina. These included well-conducted short-term toxicity studies in mice and rats fed dried spirulina at doses of up to 45 000 and 30 000 mg/kg bw per day, respectively. Assuming a phycocyanin content of 10% based on commercial dried spirulina, the doses of phycocyanin were estimated to be 4500 and 3000 mg/kg bw per day, respectively. No evidence of carcinogenicity or systemic toxicity was observed in long-term toxicity studies in rats fed spirulina extract or dried spirulina. There were no concerns regarding genotoxicity. Reproductive and developmental toxicity were not of concern based on the absence of toxicity in feeding studies conducted with dried spirulina in mice, rats and hamsters.

Expressed as phycocyanins, estimated dietary exposure from the use of spirulina extract as a food colour, based on the budget method, and exposure to spirulina extract and dried spirulina from other dietary sources including food ingredients, dietary supplements and coatings of dietary supplements was 190 mg/kg bw for a 60 kg adult and 650 mg/kg bw for a 15 kg child. The Committee concluded that this dietary exposure does not present a health concern.

The ADI “not specified” was made temporary due to the tentative nature of the specifications.

A toxicological and dietary exposure monograph was prepared.

A new tentative specifications monograph 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; and Geneva: World Health Organization. Rome: Food and Agriculture Organization of the United Nations and Geneva: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2017 (REP17/FA).

2. Glazer AN, Fang S. Chromophore content of blue-green algal phycobiliproteins. *J Biol Chem.* 1973;248(2):659–62.
3. Minic SL, Stanic-Vucinic D, Mihailovic J, Krstic M, Nikolic MR, Cirkovic Velickovic T. Digestion by pepsin releases biologically active chromopeptides from C-phycoyanin, a blue-colored biliprotein of microalga *Spirulina*. *J Proteomics.* 2016;147:132–9.
4. Schram BL, Kroes HH. Structure of phycocyanobilin. *Eur J Biochem.* 1971;19(4):581–94.
5. McCarty MF. Clinical potential of spirulina as a source of phycocyanobilin. *J Med Food.* 2007;10(4):566–70.
6. Eriksen NT. Production of phycocyanin – a pigment with applications in biology, biotechnology, foods and medicine. *Appl Microbiol Biotechnol.* 2008;80(1):1–14.
7. Ciferri O. *Spirulina*, the edible microorganism. *Microbiol Rev.* 1983;47(4):551–78.
8. Scheldeman P, Baurain D, Bouhy R, Scott M, Mühling M, Whitton BA et al. *Arthrospira* ('*Spirulina*') strains from four continents are resolved into only two clusters, based on amplified ribosomal DNA restriction analysis of the internally transcribed spacer. *FEMS Microbiol Lett.* 1999;172(2):213–22.
9. Kwei CK, Lewis D, King K, Donohue W, Nielan BA. Molecular classification of commercial *Spirulina* strains and identification of their sulfolipid biosynthesis genes. *J Microbiol Biotechnol.* 2011;21(4):359–65.
10. Romay C, Ledón N, González R. Further studies on anti-inflammatory activity of phycocyanin in some animal models of inflammation. *Inflamm Res.* 1998;47(8):334–8.
11. Naidu AK, Sarada R, Manoj G, Khan MY, Swamy MM, Viswanatha S et al. Toxicity assessment of phycocyanin – a blue colorant from blue green alga *Spirulina platensis*. *Food Biotechnol.* 1999;13(1):51–66.
12. Chen YH, Chang GK, Kuo SM, Huang SY, Hu IC, Lo YL et al. Well-tolerated *Spirulina* extract inhibits influenza virus replication and reduces virus-induced mortality. *Sci Rep.* 2016;6(24253):1–11.
13. Hutadilok-Towatana N, Reanmongkol W, Satitit S, Panichayupakaranant P, Ritthisunthorn P. A subchronic toxicity study of *Spirulina platensis*. *Food Sci Technol Res.* 2008;14(4):351–58.
14. Song L, Liu B, Zhao Y, Tian J, Qin S. Chronic toxicity study of phycocyanin on Sprague Dawley rats. *Chin Med Herald.* 2012;9(33):15–21.
15. Takemoto K. Chronic toxicity study of phycocyanin (Linablue A). Unpublished study by Saitama Medical School, Moroyama, Saitama Prefecture, Japan; 1980. Submitted to WHO by the International Association of Color Manufacturers (IACM), Washington, DC, USA.
16. Yang Y, Park Y, Cassada DA, Snow DD, Rogers DG, Lee J. In vitro and in vivo safety assessment of edible blue-green algae, *Nostoc commune* var. *sphaeroides* Kützing and *Spirulina plantensis*. *Food Chem Toxicol.* 2011;49(7):1560–4.
17. Chamorro GA, Herrera G, Salazar M, Salazar S, Ulloa V. Subchronic toxicity study in rats fed spirulina. *J Pharm Belg.* 1988;43(1):29–36.
18. Salazar M, Martinez E, Madrigal E, Ruiz LE, Chamorro GA. Subchronic toxicity study in mice fed *Spirulina maxima*. *J Ethnopharmacol.* 1998;62(3):235–41.
19. Yang Y, Kim B, Park YK, Lee JY. Effects of long-term supplementation of blue-green algae on lipid metabolism in C57BL/6J mice. *J Nutrit Health Food Sci.* 2014;1(1):1–6.

20. Vidé J, Romain C, Feillet-Coudray C, Bonafos B, Cristol JP, Fouret G et al. Assessment of potential toxicological aspects of dietary exposure to silicon-rich spirulina in rats. *Food Chem Toxicol.* 2015;80:108–13.
21. Bigagli E, Cinic L, Niccolai A, Tredici MR, Biondi N, Rodolfi L et al. Safety evaluations and lipid-lowering activity of an *Arthrospira platensis* enriched diet: a 1-month study in rats. *Food Res Int.* 2017;102:380–6.
22. Chamorro-Cevallos G. Toxicologic study of the spirulina algae pilot plant for the production of proteins (*Spirulina de Soso*, Texcoco LLP). Vienna: United Nations Industrial Development Organization (UNIDO); 1980 (UNIDO/IO 387).
23. Chamorro G, Salazar M. Dominant lethal assay of *Spirulina maxima* in male CD-1 mice after short-term and prolonged-term feeding. *J Food Prot.* 1989;52(2):125–7.
24. Chamorro G, Salazar M, Pages N. Dominant lethal study of *Spirulina maxima* in male and female rats after short-term feeding. *Phytother Res.* 1996;10:28–32.
25. Chamorro G, Salazar S, Salazar M, Pages N. [Evaluation of the teratological effect of Spirulina in hamster.] *Belg J Food Chem Biotech.* 1987;42:188–91 (in French).
26. Chamorro G, Salazar S, Favila-Castilo L, Steele C, Salazar M. Reproductive and peri- and postnatal evaluation of *Spirulina maxima* in mice. *J Appl Phycol.* 1997;9(2):107–12.
27. Chamorro G, Salazar M. Teratogenic study of Spirulina in mice. *Arch Latinoam Nutr.* 1990;40(1):86–94.
28. Salazar M, Chamorro GA, Salazar S, Steele CE. Effect of *Spirulina maxima* consumption on reproduction and peri- and postnatal development in rats. *Food Chem Toxicol.* 1996;34(4):353–9.
29. Madeira M, Cardoso C, Lopes P, Coelho D, Afonso C, Bandrra N et al. Microalgae as feed ingredients for livestock production and meat quality: A review. *Livest Sci.* 2017;205:111–21.
30. Marles RJ, Barrett ML, Barnes J, Chavez ML, Gardiner P, Ko R et al. United States pharmacopeia safety evaluation of spirulina. *Crit Rev Food Sci Nutr.* 2011;51(7):593–604.
31. Hansen SC. Conditions for use of food additives based on a budget for an acceptable daily intake. *J Food Prot.* 1979;42:429–32.

## 3.2 Revision of specifications and analytical methods

### 3.2.1 Cassia gum

Cassia gum was on the agenda at the request of the Committee at its eighty-second meeting. The specifications for cassia gum were made tentative by the eighty-second JECFA ([Annex 1](#), reference 230) pending submission of a validated method for the determination of anthraquinones. The Committee, at the current meeting, considered the analytical methods provided and included the most suitable validated method in the specifications monograph. However, this method uses chloroform for the extraction of anthraquinones. Extraction with *n*-hexane and diethyl ether resulted in poor recovery of anthraquinones. The Committee recommends that the JECFA Secretariat be notified if an alternative extraction solvent is identified.

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

### 3.2.2 Citric and fatty acid esters of glycerol

Citric and fatty acid esters of glycerol (CITREM) was on the agenda at the request of the Committee at its seventy-ninth and eighty-second meetings ([Annex 1](#), references 174 and 230), requesting a replacement method for the obsolete packed column gas chromatographic method for the determination of total citric acid, in the specifications monographs. The Committee did not receive any suitable replacement method. The specifications for CITREM were made tentative, pending a suitable validated method for the determination of total citric acid content, along with performance characteristics of the method and data on the total citric acid content in at least five batches of products currently available in commerce, determined using that method.

The Committee noted that the method for total glycerol still uses chloroform. The Committee encourages the submission of a method for total glycerol that eliminates the use of chloroform.

The Committee noted that neutralizing agents, other than sodium and potassium hydroxides, are in use for the partial or full neutralization of CITREM products intended for some food applications. The Committee noted that a request was made to extend the definition of CITREM to include the use of alternative neutralizing agents. However, the Committee emphasizes that such requests shall be made through the corresponding Codex committees.

Specifications were revised and made tentative. Specifications will be withdrawn if suitable information is not provided by December 2019.

A Chemical and Technical Assessment was prepared.

### 3.2.3 Glycerol ester of wood rosin

Glycerol ester of wood rosin (GEWR) was on the agenda of the current meeting at the request of Codex Committee on Food Additives [1] to allow the use of additional species of pine as source materials in the manufacture of GEWR. The current specifications refer to two pine species: *Pinus palustris* and *Pinus elliottii*. The Committee received information on the manufacture of GEWR from the rosin obtained from the stumps of two additional species, namely *Pinus halepensis* and *Pinus brutia*, as source materials. The total esterified abietic acids in GEWR (abietic acid, dehydroabietic acid and neoabietic acid) prepared from the four pine species range from approximately 72% to 85%. Recognizing the natural variability of the composition of wood rosin, the Committee removed the restriction to certain pine species within the specifications. The Committee further noted that this harmonizes the JECFA specifications with those of other regulatory authorities.

Identification of GEWR is based on the presence of characteristic acids. Since the specifications monograph for GEWR does not contain an assay, the Committee recommends that the JECFA Secretariat be notified upon the development and validation of an appropriate assay.

The existing specifications were revised. The Chemical and Technical Assessment was revised.

## 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 and Geneva: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2017 (REP17/FA).

### 3.2.4 Modified starches

#### General considerations

The Committee at its seventy-ninth meeting ([Annex 1](#), reference 220) recommended the separation of the combined specifications for the modified starches into 16 individual monographs (INS Nos 1400, 1401, 1402, 1403, 1404, 1405, 1410, 1412, 1413, 1414, 1420, 1422, 1440, 1442, 1450, 1451). The recommendation was based on the difficulty of revising individual specifications for any given modified starch within the existing combined specifications monograph.

The Committee at its eighty-second meeting ([Annex 1](#), reference 230) reviewed individual specifications monographs for each of the 16 modified starches and, based on the limited information received, prepared full specifications for three (INS Nos 1404, 1420, 1451) and tentative specifications for the remaining 13 (INS Nos 1400, 1401, 1402, 1403, 1405, 1410, 1412, 1413, 1414, 1422, 1440, 1442, 1450). The Committee noted that all the modified starches may also be subjected to bleaching and therefore included the appropriate purity tests in all the revised specifications. Data and information necessary to complete and revise the 13 individual tentative specifications monographs, as well as information on the method of manufacture for each of the 16 modified starches, were requested through a further call for data.

At the current meeting, the Committee reviewed data on the method of manufacture, identity and purity of all 16 modified starches.

The Committee noted the following:

- All processes are performed under similar manufacturing conditions and result in minor chemical modifications. Given the chemical and physical similarities of modified starches, the Committee at previous meetings considered a read-across approach to be appropriate for the toxicological evaluation of these substances.

- All 16 modified starches had been assigned an ADI “not specified”.
- All modified starches can be additionally bleached or fragmented; therefore, revision of the specifications of bleached or fragmented starches would require the revision of all 16 monographs.
- Microbiological specifications were not present in the existing specifications for all modified starches.
- Several specifications were common to all modified starches (such as for heavy metals content and microbiological considerations). Revision of those common specifications would affect all 16 monographs.
- Because of the wide range of products manufactured, the identification tests required to unambiguously chemically characterize each modified starch in individual specifications may be cumbersome, unavailable and unlikely to reflect market requirements.
- It may not be possible to publish identification tests based on market requirements without unduly revealing proprietary information.

Based on the points noted above, individual specifications for several modified starches may remain tentative for an indefinite period or may need to be withdrawn.

The Committee therefore recommended that a new approach to the specifications monographs be introduced to account for the chemical similarity between all modified starches, their functional diversity, the variety of chemicals used in their manufacture and the corresponding diversity of impurities.

The Committee recommended that all modified starches be included in a modular monograph titled “Modified Starches” that contains common requirements (“General specifications for modified starches”) consisting of specifications that apply to all 16 modified starches (INS Nos 1400, 1401, 1402, 1403, 1404, 1405, 1410, 1412, 1413, 1414, 1420, 1422, 1440, 1442, 1450, 1451) and annexes with specifications applicable to each individual modified starch based on the treatment(s) received. The annexes are as follows:

- Annex 1 – Fragmentation
- Annex 2 – Bleaching
- Annex 3 – Esterification and/or crosslinking with phosphorus-containing compounds
- Annex 4 – Acetylation
- Annex 5 – Oxidation
- Annex 6 – Esterification with octenyl succinic anhydride
- Annex 7 – Etherification with propylene epoxide
- Annex 8 – Esterification and crosslinking with adipic anhydride

Table 10  
**List of modified starches considered and applicable annexes**

Modified starch	INS No.	Annex
Dextrin roasted starch	1400	1
Acid treated starch	1401	1
Alkaline treated starch	1402	1
Bleached starch	1403	2
Oxidized starch	1404	5
Enzyme-treated starch	1405	1
Monostarch phosphate	1410	3
Distarch phosphate	1412	3
Phosphated distarch phosphate	1413	3
Acetylated distarch phosphate	1414	3, 4
Starch acetate	1420	4
Acetylated distarch adipate	1422	4, 8
Hydroxypropyl starch	1440	7
Hydroxypropyl distarch phosphate	1442	3, 7
Starch sodium octenyl succinate	1450	6
Acetylated oxidized starch	1451	4, 5

INS: International Numbering System for Food Additives

Each modified starch should fulfil the specification requirements of the General specifications as well as the specification requirements of all the annexes applicable to it.

The Committee determined that, using this approach, revisions made to the section “General specifications for modified starches” would affect all the modified starches. However, annexes can be revised individually and the revisions would only affect the modified starches mentioned in that annex. A tentative status prompted by information missing from the General specifications would affect all 16 modified starches. A tentative status of an annex would only affect the status of modified starches covered by that annex.

In response to the considerations noted above, the Committee drafted a new modular specifications monograph titled “Modified starches” consisting of an explanatory introduction, “General specifications for modified starches”, and eight annexes. The new modular specifications monograph for modified starches is to be included in FAO Monographs 22, and will replace the 16 existing individual specifications for modified starches (INS Nos 1400, 1401, 1402, 1403, 1404, 1405, 1410, 1412, 1413, 1414, 1420, 1422, 1440, 1442, 1450, 1451).

## Technical considerations

In line with previous decisions, the Committee noted that the processes of starch modification should not result in any additional contamination from heavy metals and therefore recommended that the limit for lead included in the General specifications be decreased from 2 to 0.2 mg/kg. This change is in line with the limit for lead in grains and cereals currently contained in the Codex General Standard for Contaminants in Food [1].

Starch sodium octenyl succinate (INS No. 1450) was on the agenda of the eighty-second JECFA meeting at the request of CCFA at its Forty-seventh Session [2] to assess the data on the levels of lead when the additive is used in infant formula and formula for special medical purposes for infants and to consider a specific limit in the specifications.

At the current meeting, the Committee received data for the lead content from 12 batches of starch sodium octenyl succinate for use in infant formula and formula for special medical purposes intended for infants. The limit of lead for starch sodium octenyl succinate for use in infant formula and formula for special medical purposes intended for infants was set to 0.1 mg/kg in the General specifications. This limit is in line with the recommendation from the seventy-ninth JECFA that the introduction of lead limits of 0.1 mg/kg for starch sodium octenyl succinate would result in this additive not exceeding the maximum limit for lead in the final infant formula (i.e. 0.01 mg/kg) if the additive were included in infant formula at the maximum use level reviewed by JECFA.

The Committee further noted that during the manufacture of hydroxypropyl starch (INS No. 1440) and hydroxypropyl distarch phosphate (INS No. 1442), propylene chlorohydrins can occur as impurities. The current specification for propylene chlorohydrins is “Not more than 1 mg/kg”. Based on information available in the literature [3] and the limited data received, the Committee requested additional data and a suitable method for the determination of propylene chlorohydrins in these modified starches in order to consider lowering this limit.

The methods for the determination of free adipic acid and adipate groups, residual vinyl acetate, free octenyl succinic acid and octenyl succinate esters were revised and a method for the determination of propylene chlorohydrins was added. Should more suitable methods become available, the Committee recommends that these methods be communicated to the JECFA secretariat.

The Committee requests suitable microbiological acceptance criteria and supporting data for all modified starches. Information required in the annexes is summarized in [Table 11](#).



Table 11  
Information required in the annexes

Annex	Modification	Starches	Information required
1	Minor fragmentation	INS No. 1400: Dextrin roasted starch INS No. 1401: Acid treated starch INS No. 1402: Alkaline treated starch INS No. 1405: Enzyme-treated starch All modified starches that are additionally fragmented.	A suitable method for dispersion and a method for reducing sugars and data on at least 5 representative batches using the method(s) from each of the fragmentation processes
2	Bleaching	INS No. 1403: Bleached starch All modified starches if additionally bleached.	Suitable method(s) for the determination of residual reagents and data on at least 5 representative batches using the method(s)
3	Esterification and/or crosslinking with phosphorus-containing compounds	INS No. 1410: Monostarch phosphate INS No. 1412: Distarch phosphate INS No. 1413: Phosphated distarch phosphate INS No. 1414: Acetylated distarch phosphate INS No. 1442: Hydroxypropyl distarch phosphate	A suitable method for identification of crosslinking and data on at least 5 representative batches of crosslinked and non-crosslinked starches
4	Acetylation	INS No. 1420: Starch acetate INS No. 1414: Acetylated distarch phosphate INS No. 1422: Acetylated distarch adipate INS No. 1451: Acetylated oxidized starch	Currently no additional information required
5	Oxidation	INS No. 1404: Oxidized starch INS No. 1451: Acetylated oxidized starch	A suitable method for determination of residual hypochlorite and data on at least 5 representative batches using the method
6	Esterification with octenyl succinic anhydride	INS No. 1450: Starch sodium octenyl succinate	Currently no additional information required
7	Etherification with propylene epoxide	INS No. 1440: Hydroxypropyl starch INS No. 1442: Hydroxypropyl distarch phosphate	A suitable method for the determination of propylene chlorohydrin with detection limit lower than 0.1 mg/kg and data on at least 5 representative batches of hydroxypropyl starch using the method
8	Crosslinking with adipic anhydride	INS No. 1422: Acetylated distarch adipate	A suitable method for identification of crosslinking and data on at least 5 representative batches of crosslinked and non-crosslinked starches Levels of free adipic acid in at least 5 representative batches

INS: International Numbering System for Food Additives; No.: number

## References

1. General standard for contaminants and toxins in food and feed. Rome: Food and Agriculture Organization of the United Nations, Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 1995 (Codex Stan 193-1995).
2. Report of the Forty-seventh Session of the Codex Committee on Food Additives, Xi'an, China, 23–27 March 2015. Rome: Food and Agriculture Organization of the United Nations; and Geneva: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2015 (REP15/FA).
3. Scientific Opinion on the re-evaluation of oxidised starch (E 1404), monostarch phosphate (E 1410), distarch phosphate (E 1412), phosphated distarch phosphate (E 1413), acetylated distarch phosphate

(E 1414), acetylated starch (E 1420), acetylated distarch adipate (E 1422), hydroxypropyl starch (E 1440), hydroxypropyl distarch phosphate (E 1442), starch sodium octenyl succinate (E 1450), acetylated oxidised starch (E 1451) and starch aluminium octenyl succinate (E 1452) as food additives. EFSA J. 2017;15(10):4911 [96 pp.] doi:10.2903/j.efsa.2017.4911.

## 4. Flavouring agents

### 4.1 Toxicological evaluation and exposure assessment

#### 4.1.1 Alicyclic primary alcohols, aldehydes, acids and related esters

##### Introduction

The Committee evaluated an additional three flavouring agents belonging to the group of alicyclic primary alcohols, aldehydes, acids and related esters. These three flavouring agents have not previously been evaluated by the Committee. The Committee also re-evaluated six previously evaluated flavouring agents in this group.

The Committee previously evaluated 26 members of this group of flavouring agents at its fifty-ninth meeting ([Annex 1](#), reference 160) and 11 members of this group at its seventy-third meeting ([Annex 1](#), reference 202). The Committee concluded that all 37 flavouring agents were of no safety concern at estimated dietary exposures.

The three additional flavouring agents in this group are the mixture of 1-vinyl-3-cyclohexenecarbaldehyde and 4-vinyl-1-cyclohexenecarbaldehyde (No. 2253); (1-methyl-2-(1,2,2-trimethylbicyclo[3.1.0]hex-3-ylmethyl)cyclopropyl) methanol (No. 2254); and ( $\pm$ )-bicyclo[2.2.1]hept-5-ene-2-carboxylic acid, ethyl ester (No. 2255). These three flavouring agents have not been reported to occur as natural components of foods.

The six previously evaluated flavouring agents in this group that were re-evaluated at the present meeting are *p*-mentha-1,8-dien-7-al (perillaldehyde; No. 973), *p*-mentha-1,8-dien-7-ol (No. 974), *p*-mentha-1,8-dien-7-yl acetate (No. 975), formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene (No. 980), myrtenol (No. 981) and myrtenyl acetate (No. 982). These six flavouring agents are all reported to occur as natural components of foods, including bergamot oil, blackberry, black tea, cumin, ginger, grapefruit oil, kabosu oil, kumquat oil, lamb's lettuce, lemon peel oil, lime oil, mandarin oil, orange juice and oil, pepper, peppermint, pistachio, spearmint, thyme, yuzu oil and other foods [1].

Two of the six previously evaluated flavouring agents, *p*-mentha-1,8-dien-7-al (No. 973) and formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene (No. 980), contain an  $\alpha,\beta$ -unsaturated aldehyde group that is considered to be a structural alert for genotoxicity [2]. The remaining four re-evaluated flavouring agents in this group are not  $\alpha,\beta$ -unsaturated aldehydes, but are structurally related to Nos 973 and 980. Additional *in vitro* and *in vivo* genotoxicity data were available for all six of these previously evaluated flavouring agents. Also, one of the two major components of an additional flavouring agent evaluated by the Committee at the present meeting (No. 2253, which is a mixture of 1-vinyl-3-

cyclohexenecarbaldehyde and 4-vinyl-1-cyclohexenecarbaldehyde) contains an  $\alpha,\beta$ -unsaturated aldehyde group. In vitro and in vivo genotoxicity data on No. 2253 were also available for evaluation.

The evaluations of the three additional members of this group and the re-evaluation of six previously considered flavouring agents in this group were conducted using the Procedure for the Safety Evaluation of Flavouring Agents ([Annex 1](#), reference 230).

### Assessment of dietary exposure

The total annual volumes of production of the nine flavouring agents belonging to the group of alicyclic primary alcohols, aldehydes, acids and related esters are 36 kg in Europe, 34 kg in the USA, 3030 kg in Japan and 28 kg in Latin America [3, 4]. More than 97% of the annual production volume in Japan is accounted for by *p*-mentha-1,8-dien-7-al (No. 973). More than 97% of the annual production volume in Europe and the USA are accounted for by three flavouring agents: *p*-mentha-1,8-dien-7-al (No. 973); *p*-mentha-1,8-dien-7-yl acetate (No. 975); and formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene (No. 980). More than 96% of the annual production volume in Latin America is accounted for by three flavouring agents, *p*-mentha-1,8-dien-7-al (No. 973), formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene (No. 980) and myrtenol (No. 981).

Annual volumes of production in the USA for the three additional flavouring agents belonging to the group of alicyclic primary alcohols, aldehydes, acids and related esters are 0.4 kg each for Nos 2253 and 2254 and 0.1 kg for No. 2255. Annual production volume for these three flavouring agents in Europe, Japan or Latin America is reported as 0 kg [3, 4].

Dietary exposures were estimated using both the single-portion exposure technique (SPET) and the maximized survey-derived intake (MSDI) method, and the higher of the two values for each flavouring agent is reported in [Table 12](#). The SPET and MSDI method values are in the range of 30–1500 and 0.01–780  $\mu\text{g}/\text{day}$ , respectively, with the SPET yielding the highest estimate for each flavouring agent. The estimated daily dietary exposure was highest for the mixture of 1-vinyl-3-cyclohexenecarbaldehyde and 4-vinyl-1-cyclohexenecarbaldehyde (No. 2253) (1500  $\mu\text{g}/\text{day}$ , the SPET value obtained for non-alcoholic beverages).

### Absorption, distribution, metabolism and excretion

Information on the ADME of this group of flavouring agents was described in the reports of the fifty-ninth and seventy-third meetings ([Annex 1](#), references 160 and 202). No additional information was available for this meeting.

### Consideration of genotoxicity data

The Committee considered new genotoxicity data on six members of this group. The six members were evaluated at the fifty-ninth meeting ([Annex 1](#), reference 160) and re-evaluated at the present meeting: *p*-mentha-1,8-dien-7-al (No. 973), *p*-mentha-1,8-dien-7-ol (No. 974), *p*-mentha-1,8-dien-7-yl acetate (No. 975), formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene (No. 980), myrtenol (No. 981) and myrtenyl acetate (No. 982). Two of these six flavouring agents, *p*-mentha-1,8-dien-7-al (No. 973) and formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene (No. 980), contain an  $\alpha,\beta$ -unsaturated aldehyde group considered to be a structural alert for genotoxicity [2].

The Committee considered the new genotoxicity data for No. 973 and noted that there were reproducible positive results in a reverse mutation assay in the TA98 strain of *Salmonella typhimurium* [5]. In addition, a study in rats investigated micronucleus induction in bone marrow and DNA damage in liver and duodenum (comet assay) [6, 7]. In this GLP-compliant study, the micronucleus assay was conducted according to OECD guideline 474 while the comet assay was conducted in accordance with published guidelines developed by an expert working group [8]. The results of the micronucleus assay indicated that *p*-mentha-1,8-dien-7-al (No. 973) did not induce an increase in micronucleated polychromatic erythrocytes. Although the comet assay did not indicate DNA damage in duodenum compared to the negative control, a 3-fold increase in DNA strand breaks (statistically significant,  $P < 0.001$ ) was observed in liver at the highest dose tested (700 mg/kg bw per day), and there was a dose-dependent trend in the response ( $P < 0.001$ ). Based on these new data, the Committee concluded that there are concerns for potential genotoxicity for *p*-mentha-1,8-dien-7-al (No. 973).

For No. 980, new in vitro reverse mutation [9, 10] and micronucleus induction [11, 12] studies were negative. Also available were new in vivo assays on micronucleus induction in bone marrow and DNA damage in liver and duodenum [13] conducted in the same laboratory and using the same protocols as used for the study on No. 973. The results of the micronucleus assay indicated no increase in micronucleated polychromatic erythrocytes and the results of the comet assay indicated no DNA damage in duodenum or liver. The Committee concluded that there are no concerns for potential genotoxicity for formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene (No. 980).

The remaining four re-evaluated flavouring agents in this group are not  $\alpha,\beta$ -unsaturated aldehydes but are structurally related to Nos 973 and 980. Additional in vitro and in vivo genotoxicity data were available on these flavouring agents for evaluation at the present meeting. None of these studies indicated potential for genotoxicity.

One of the two major components of an additional flavouring agent evaluated by the Committee at the present meeting (No. 2253, which is a mixture of 1-vinyl-3-cyclohexenecarbaldehyde and 4-vinyl-1-cyclohexenecarbaldehyde) also contains an  $\alpha,\beta$ -unsaturated aldehyde group, and in vitro and in vivo genotoxicity data on No. 2253 were available for evaluation. In a bacterial reverse mutation assay, No. 2253 was negative in all five strains tested in both the presence and absence of metabolic activation [14]. In an in vitro mammalian chromosomal aberration assay, No. 2253 induced an increase in the percentage of aberrant cells [15]. However, a GLP-compliant in vivo micronucleus induction assay in mice, conducted in accordance with OECD guideline 474, was negative [16]. The Committee concluded that there are no concerns for potential genotoxicity for the mixture of 1-vinyl-3-cyclohexenecarbaldehyde and 4-vinyl-1-cyclohexenecarbaldehyde (No. 2253).

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

**Step 1.** Genotoxicity data on the three additional flavouring agents in this group (Nos 2253–2255) and five of the six re-evaluated flavouring agents in this group do not indicate that these flavouring agents have the potential for genotoxicity. For one of the re-evaluated flavouring agents, *p*-mentha-1,8-dien-7-al (No. 973) the Committee concluded that there were concerns for genotoxicity. Therefore, No. 973 was not further considered using the Procedure for the Safety Evaluation of Flavouring Agents.

**Step 2.** In applying the Procedure, the Committee assigned six flavouring agents (Nos 974, 975, 980–982 and 2253) to structural class I, one flavouring agent (No. 2254) to structural class II and one flavouring agent (No. 2255) to structural class III [17].

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

**Step 4.** The highest estimated dietary exposures for all eight flavouring agents were below the threshold of toxicological concern applicable to each flavouring agent. The Committee therefore concluded that these eight flavouring agents would not pose a safety concern at current estimated dietary exposures.

**Table 12** summarizes the evaluations of the eight flavouring agents belonging to this group of alicyclic primary alcohols, aldehydes, acids and related esters that were considered at the present meeting (Nos 974, 975, 980–982 and 2253–2255).

### Consideration of combined intakes from use as flavouring agents

The Committee previously considered the potential combined intakes for this group of alicyclic primary alcohols, aldehydes, acids and related esters and did not identify any safety concerns. The three additional flavouring agents in this

Table 12

**Summary of the results of the safety evaluations of alicyclic primary alcohols, aldehydes, acids and related esters used as flavouring agents<sup>a,b,c,d</sup>**


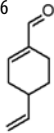
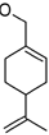
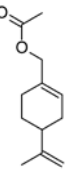

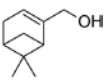
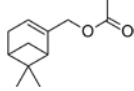
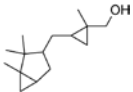
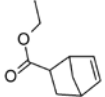
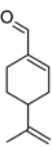
Flavouring agent	No.	CAS no. and structure	Step 4 <sup>e</sup> Does the highest dietary exposure estimate exceed the threshold of toxicological concern?	Conclusion based on current estimated dietary exposure	
<b>Structural class I</b>					
Mixture of 1-vinyl-3-cyclohexenecarbaldehyde and 4-vinyl-1-cyclohexenecarbaldehyde	2253	1049017-63-1 	1049017-68-6 	No, SPET: 1 500	No safety concern
<i>p</i> -Mentha-1,8-dien-7-ol	974	536-59-4 		No, SPET: 1 000	No safety concern
<i>p</i> -Mentha-1,8-dien-7-yl acetate	975	15111-96-3 		No, SPET: 1 000	No safety concern
Formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene	980	564-94-3 		No, SPET: 1 000	No safety concern
Myrtenol	981	515-00-4 		No, SPET: 1 000	No safety concern
Myrtenyl acetate	982	1079-01-2 		No, SPET: 1 000	No safety concern
<b>Structural class II</b>					
(1-Methyl-2-(1,2,2-trimethylbicyclo[3.1.0]hex-3-ylmethyl)cyclopropyl)methanol	2254	198404-98-7 		No, SPET: 30	No safety concern
<b>Structural class III</b>					
(±)-Bicyclo[2.2.1]hept-5-ene-2-carboxylic acid, ethyl ester	2255	10138-32-6 		No, SPET: 60	No safety concern

Table 12 (continued)

Flavouring agent	No.	CAS no. and structure	Step 4 <sup>e</sup> Does the highest dietary exposure estimate exceed the threshold of toxicological concern?	Conclusion based on current estimated dietary exposure
<b>Flavouring agent excluded at Step 1 of the Procedure</b>				
<i>p</i> -Mentha-1,8-dien-7-al (perillaldehyde)	973	2111-75-3 	NA	Genotoxicity data for <i>p</i> -mentha-1,8-dien-7-al raise concerns for potential genotoxicity

bw: body weight; CAS: Chemical Abstracts Service; MSDI: maximized survey-derived intake; NA: not applicable; No.: number; NOAEL: no-observed-adverse-effect level; SPET: single-portion exposure technique

<sup>a</sup> Thirty-seven flavouring agents in this group were previously evaluated by the Committee (Annex 1, references 160 and 202). Six of those flavouring agents (Nos 973–975 and 980–982) and three additional flavouring agents in this group (Nos 2253–2255) were also evaluated at the present meeting.

<sup>b</sup> Step 1: Genotoxicity data for one of the nine flavouring agents in this group, *p*-mentha-1,8-dien-7-al (No. 973), raise concerns for potential genotoxicity. Genotoxicity data for the remaining eight flavouring agents in this group do not indicate potential genotoxicity.

<sup>c</sup> Step 2: The additional flavouring agents in this group are in structural class I (No. 2253), class II (No. 2254) and class III (No. 2255). The five re-evaluated flavouring agents in this group are in structural class I.

<sup>d</sup> Step 3: Dietary exposures were estimated using both the SPET and the MSDI method, and the higher of the two values for each flavouring agent is reported in Table 12. The SPET gave the highest estimate for each flavouring agent. Dietary exposure values are expressed in µg/day.

<sup>e</sup> Step 4: The thresholds for human dietary exposure for structural classes I, II and III are 1800, 540 and 90 µg/day, respectively.

group (Nos 2253–2255) have low MSDI values (0.01–0.04 µg/day) and therefore would make a negligible contribution to the combined intake of this group.

### Consideration of additional data on previously evaluated flavouring agents

In addition to new genotoxicity data on the six flavouring agents that were re-evaluated at the present meeting, the Committee considered additional data on several other previously evaluated flavouring agents in this group. Acute toxicity data were evaluated for Nos 961, 967, 977 and 981, and studies of genotoxicity were evaluated for Nos 967, 977–979 and 984. These new toxicological data support the conclusions of previous Committee evaluations that these flavouring agents are not safety concerns.

### Conclusions

In previous evaluations of flavouring agents in this group of alicyclic primary alcohols, aldehydes, acids and related esters, studies of hydrolysis; ADME; acute, short-term and long-term toxicity; and genotoxicity were available. None of the 37 previously evaluated flavouring agents raised safety concerns.



At the present meeting, the Committee concluded that the three flavouring agents (Nos 2253–2255) that are additions to the group of alicyclic primary alcohols, aldehydes, acids and related esters, would not give rise to safety concerns at current estimated dietary exposures.

The Committee also concluded that five of the six previously evaluated flavouring agents in this group (Nos 974, 975 and 980–982) that were re-evaluated at the present meeting do not give rise to safety concerns. For one of the re-evaluated flavouring agents, *p*-mentha-1,8-dien-7-al (perillaldehyde; No. 973), the Committee concluded that there were concerns for potential genotoxicity. Therefore No. 973 was not further considered using the Procedure.

An addendum to the monograph was prepared.

## References

1. Volatile compounds in food, version 16.4 [database]. Zeist, the Netherlands: Triskelion B.V.; 2017 (<http://www.vcf-online.nl/VcfHome.cfm>, accessed 5 January 2018).
2. Eder E, Scheckenbach S, Deininger C, Hoffman C. The possible role of alpha, beta-unsaturated carbonyl compounds in mutagenesis and carcinogenesis. *Toxicol Lett.* 1993;67(1–3):87–103.
3. 2015 IOFI Global Poundage Survey. International Organization of the Flavor Industry, Brussels; 2017a. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
4. Interim inquiry on volume use and added use levels for flavoring agents to be presented at the JECFA 86th meeting. Private communication to the International Organization of the Flavor Industry, Brussels; 2017b. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
5. Bowen R. Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium*. Covance Laboratories Ltd, North Yorkshire, England, United Kingdom; 2011. Study no. 8221207. Unpublished report to the International Organization of the Flavor Industry. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
6. Beevers C. *p*-Mentha-1,8-dien-7-al: Combined bone marrow micronucleus test and comet assay in the liver of treated rats. Covance Laboratories Ltd, North Yorkshire, England, United Kingdom; 2014a. Study no. 8288455. Unpublished report to the International Organization of the Flavor Industry. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
7. Beevers C. *p*-Mentha-1,8-dien-7-al: Analysis of comet slides from Covance study 8288455. Covance Laboratories Ltd, North Yorkshire, England, United Kingdom; 2014b. Study no. 8307194. Unpublished report to the International Organization of the Flavor Industry. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
8. Burlinson B, Tice RR, Speit G, Agurell E, Brendler-Schwaab SY, Collins AR et al. Fourth International Workgroup on Genotoxicity testing: results of the in vivo comet assay workgroup. *Mutat Res.* 2007;627(1):31–5.
9. McGarry S. Myrtenal: Bacterial reverse mutation assay. Covance Laboratories Ltd, North Yorkshire, England, United Kingdom; 2016b. Study no. 8332788. Unpublished report to the International Organization of the Flavor Industry. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.

10. Dakoulas E. 2-Formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene (CAS No. 564-94-3): Bacterial reverse mutation assay. BioReliance Corporation, Maryland, USA; 2017. Study no. AE67ZC.503.BTL. Unpublished report to the Research Institute for Fragrance. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
11. McGarry S. Myrtenal: In vitro human lymphocyte micronucleus assay. Covance Laboratories Ltd, North Yorkshire, England, United Kingdom; 2016a. Study no. 8332792. Unpublished report to the International Organization of the Flavor Industry. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
12. Lloyd M. Myrtenal: In vitro human lymphocyte micronucleus assay. Covance Laboratories Ltd, North Yorkshire, England, United Kingdom; 2017. Study no. 8351223. Unpublished report to the International Organization of the Flavor Industry. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
13. Pant K. In vivo mammalian erythrocyte micronucleus assay and mammalian alkaline comet assay in rats. BioReliance Corporation, Maryland, USA; 2018. Study no. AF08WZ.433M.BTL. Unpublished report to the International Organization of the Flavor Industry. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
14. Sokolowski A. *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay with GR-50-0091. Harlan Laboratories Ltd, Rossdorf, Germany; 2011. Study no. 1406304. Unpublished report to the Research Institute for Fragrance Materials, Woodcliff Lake, NJ, USA. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
15. Hall C. In vitro chromosome aberration test in Chinese hamster V79 cells with G-50-0091. Harlan Laboratories Ltd, Rossdorf, Germany; 2011a. Study no. 1406305. Unpublished report to the Flavor and Extract Manufacturers Association, Washington, DC, USA. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
16. Hall C. Micronucleus assay in bone marrow cells of the mouse with [redacted]. Harlan Laboratories Ltd, Rossdorf, Germany; 2011b. Study no. 1439400. Unpublished report to the Flavor and Extract Manufacturers Association, Washington, DC, USA. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
17. Cramer GM, Ford RA, Hall RL. Estimation of toxic hazard – a decision tree approach. *Food Cosmet Toxicol.* 1978;16(3):255–76.

#### 4.1.2 Carvone and structurally related substances

##### Introduction

The Committee evaluated five flavouring agents belonging to the previously evaluated group of carvone and structurally related substances. The Committee re-evaluated two flavouring agents, (+)-carvone (No. 380.1; *d*-carvone) and (–)-carvone (No. 380.2; *l*-carvone), and evaluated three additional flavouring agents. These three additional flavouring agents included two esters, pinocarvyl isobutyrate (No. 2242) and carvyl palmitate (No. 2243), and one alicyclic secondary alcohol, 6-hydroxycarvone (No. 2244). The evaluations were conducted using the Procedure for the Safety Evaluation of Flavouring Agents ([Annex 1](#), reference

230). (+)-Carvone (No. 380.1) and (–)-carvone (No. 380.2) were re-evaluated because of new data had become available.

The Committee previously evaluated nine members of this group of flavouring agents at its fifty-first meeting ([Annex 1](#), reference 137). The Committee concluded that all nine flavouring agents were of no safety concern at estimated dietary exposures.

Carvone (Nos 380.1 and 380.2) was evaluated at the eleventh meeting ([Annex 1](#), reference 14) at which a conditional<sup>3</sup> ADI of 0–1.25 mg/kg bw for the (+)- and (–)-enantiomers was established. At the twenty-third meeting ([Annex 1](#), reference 50), a temporary ADI of 0–1 mg/kg bw was established for (+)- and (–)-carvone. This temporary ADI was extended at the twenty-fifth, twenty-seventh, thirtieth and thirty-third meetings ([Annex 1](#), references 56, 62, 73 and 83). At its thirty-seventh meeting, the Committee determined that the (+)- and (–)-enantiomers of carvone should be evaluated separately ([Annex 1](#), reference 94). The Committee established an ADI for (+)-carvone of 0–1 mg/kg bw per day based on a no-observed-effect level (NOEL) of 93 mg/kg bw per day from a 3-month toxicity study in rats. The temporary ADI for (–)-carvone was not extended because insufficient data were available for the toxicological evaluation of this enantiomer. The Committee at its fifty-first meeting maintained the ADI of 0–1 mg/kg bw for (+)-carvone (No. 380.1) ([Annex 1](#), reference 137).

Two of the five flavouring agents, (+)-carvone (No. 380.1) and (–)-carvone (No. 380.2), have been reported to occur naturally in foods, mainly botanicals. (+)-Carvone (No. 380.1) has been reported in *Carum* (caraway) and *Anethum* (dill). (–)-Carvone (No. 380.2) has been reported in the oils of *Mentha* (spearmint) [2].

A comprehensive literature search for toxicological data was performed in Scopus; no additional relevant references were identified.

### Assessment of dietary exposure

The total annual volume of production of the three new flavouring agents belonging to the group of carvone and structurally related substances is 2 kg in Japan [3, 4]. The total production volume for the flavouring agents presented for re-evaluation, (+)-carvone (No. 380.1) and (–)-carvone (No. 380.2), is 48 300 kg in Europe, 53 700 kg in the USA, 1780 kg in Japan and 6340 kg in Latin America [3, 4]. Separate production volumes for the two enantiomers are not available.

Dietary exposure was estimated using both the SPET and the MSDI method, with the highest values reported in [Table 13](#). The estimated daily dietary exposure is highest for carvone (Nos 380.1 and 380.2) at 37 800 µg/day, the SPET

<sup>3</sup> "Conditional ADI is a term no longer used by JECFA to signify a range above the 'unconditional ADI', which may signify an acceptable intake when special problems, different patterns of dietary intake, and special groups of the population that may require consideration are taken into account." [1]

value obtained from beer and malt beverages. The MSDI values for carvone range from 350 to 5573  $\mu\text{g}/\text{day}$  for the four regions. For the other flavouring agents, the estimated daily dietary exposures range from 0.05 to 0.3  $\mu\text{g}/\text{day}$  (MSDI values) and from 3 to 450  $\mu\text{g}/\text{day}$  (SPET values).

### Absorption, distribution, metabolism and excretion

Relevant information apart from that described in the monographs of the eleventh, twenty-third, thirty-seventh and fifty-first meetings ([Annex 1](#), references 15, 51, 95, 138) was available on the ADME of the flavouring agents belonging to the group of carvone and structurally related substances.

In general, carboxylesterases or esterases catalyse the hydrolysis of esters to their corresponding alcohol and carboxylic acid. The carvyl esters in this group (Nos 2242 and 2243) can be expected to readily hydrolyse to pinocarveol (No. 1403) and carveol (No. 974), respectively, and their respective carboxylic acids. Carvone (Nos 380.1 and 380.2) is metabolized to carveol, dihydrocarveol, carvonic acid, dihydrocarvonic acid and uroterponolone in humans [5]. Oxidation of (+)- or (–)-carvone by human or rat liver microsomes is stereospecific; (+)-carvone is oxidized exclusively to (+)-carveol and (–)-carvone to (–)-carveol, with (–)-carvone having a significantly higher affinity for microsomal enzymes (lower apparent  $K_m$ ). Only (–)-carveol is converted to a glucuronide conjugate by human or rat liver microsomes [6]. This is expected to impact the toxicity profiles of the enantiomers and their metabolites. 6-Hydroxycarvone is expected to undergo conjugation with glucuronic acid or glutathione followed by excretion.

### Flavouring agents not evaluated according to the Procedure for the Safety Evaluation of Flavouring Agents

Exposure to (+)-carvone (No. 380.1) and (–)-carvone (No. 380.2) occurs from different food and non-food sources as well as from their use as flavouring agents. Both enantiomers of carvone occur naturally in food and are used in one or more of the following applications: pesticides, feed additives, veterinary products, personal care products, natural insect repellents, food supplements and herbal medicinal products [2, 7].

For the current re-evaluation of carvone (Nos 380.1 and 380.2), additional biochemical data and studies of acute toxicity and genotoxicity were submitted by the flavour industry. The Scientific Committee of the EFSA published an evaluation of carvone based on data from the flavour and pesticide industries. An ADI of 0.6 mg/kg bw per day for (+)-carvone was established, but an ADI for (–)-carvone could not be established due to lack of data. The highest level of aggregated exposure to (+)-carvone was at the level of the ADI for (+)-carvone. The highest level of aggregated exposure to (–)-carvone was 3-fold higher than that of (+)-carvone [7].

The Committee previously established an ADI for (+)-carvone of 0–1 mg/kg bw per day based on a NOEL of 93 mg/kg bw per day from a 3-month toxicity study in rats [8]. At the current meeting, the Committee noted that when identifying the NOEL, no correction was made for the 5-day (rather than 7-day) dosing scheme. Shortly before the meeting, data from the pesticide industry (studies of acute toxicity, short-term toxicity and a 2-generation study) were made available to the Committee by the sponsor. The Committee considered these data and concluded that a review of the ADI for (+)-carvone is recommended based on the evaluation of all biochemical and toxicological data. Also, additional data are needed for an exposure assessment for the oral exposure to (+)-carvone from all sources to complete the re-evaluation of (+)-carvone (No. 380.1).

The Committee previously concluded that the ADI for (+)-carvone could not be extended to (–)-carvone because insufficient data were available for the toxicological evaluation of this enantiomer (Annex 1, reference 94). Data are also needed in an oral exposure assessment to (–)-carvone from all sources to complete the re-evaluation of (–)-carvone (No. 380.2).

The Committee therefore did not re-evaluate (+)-carvone (No. 380.1) and (–)-carvone (No. 380.2) according to the Procedure for the Safety Evaluation of Flavouring Agents at the current meeting.

### 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 2242–2244) in this group. Chemical-specific genotoxicity data on flavouring agents previously evaluated within this group do not indicate that the flavouring agents in this group have the potential to be genotoxic.

**Step 2.** In applying the Procedure for the Safety Evaluation of Flavouring Agents to Nos 2242–2244, the Committee assigned Nos 2242 and 2243 to structural class I and No. 2244 to structural class III [9].

**Steps 3 and 4.** The highest estimated dietary exposures of Nos 2242–2244 are below their respective thresholds of toxicological concern (i.e. 1800 µg/day for structural class I and 90 µg/day for structural class III). The Committee therefore concluded that these three flavouring agents would not pose a safety concern at current estimated dietary exposures.

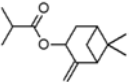
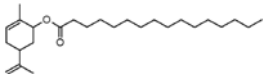
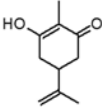
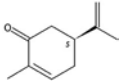
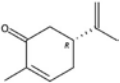
Table 13 summarizes the evaluations of the three flavouring agents (Nos 2242–2244) in the group of carvone and structurally related substances.

### Consideration of combined intakes from use as flavouring agents

The three additional flavouring agents in the group of carvone and structurally related substances have low MSDIs (0.05–0.3 µg/day). The Committee concluded that consideration of combined intakes is not necessary, because the additional

Table 13

**Summary of the results of the safety evaluations of carvone and structurally related substances used as flavouring agents<sup>a,b</sup>**

Flavouring agent	No.	CAS no. and structure	Step 4 <sup>c</sup> Does the highest dietary exposure estimate exceed the threshold of toxicological concern?	Conclusion based on current estimated dietary exposure
<b>Structural class I</b>				
Pinocarvyl isobutyrate	2242	929116-08-5 	No, SPET: 450	No safety concern
Carvyl palmitate	2243	929222-96-8 	No, SPET: 3	No safety concern
<b>Structural class III</b>				
6-Hydroxycarvone	2244	51200-86-3 	No, SPET: 4	No safety concern
<b>Flavouring agents not evaluated according to the Procedure</b>				
(+)-Carvone	380.1	2244-16-8 	A review of the ADI is recommended based on an evaluation of all biochemical and toxicological data. Also, data are needed for an assessment of oral exposure to (+)-carvone from all sources to complete the evaluation for (+)-carvone.	
(-)-Carvone	380.2	6485-40-1 		Additional toxicological data on (-)-carvone are necessary. Also, data are needed for an assessment of oral exposure to (-)-carvone from all sources to complete the evaluation for (-)-carvone.

ADI: acceptable daily intake; CAS: Chemical Abstracts Service; no.: number; SPET: single-portion exposure technique

<sup>a</sup> Nine flavouring agents in this group were previously evaluated by the Committee (Annex 1, reference 137).

<sup>b</sup> Step 2: Two additional flavouring agents in this group are in structural class I and one additional flavouring agent in this group is in structural class III.

<sup>c</sup> The threshold of toxicological concern for human dietary exposure for structural class I is 1800 µg/day and for structural class III is 90 µg/day. All dietary exposure values are expressed in µg/day. The dietary exposure values listed represent the highest estimated daily dietary exposures calculated using the SPET.

flavouring agents would not contribute significantly to the combined intake of this flavouring group.

### Consideration of additional data on previously evaluated flavouring agents

In the previous evaluations of substances in this group of carvone and structurally related substances, studies of biochemistry, acute toxicity, short-term and long-term toxicity and genotoxicity were available ([Annex 1](#), references 15, 51, 95 and 138). None of the nine flavouring agents of this group raised safety concerns.

Besides data on carvone (see the section “Flavouring agents not evaluated according to the Procedure for the Safety Evaluation of Flavouring Agents”), additional biochemical data on carveol (No. 381), a study of acute toxicity with *p*-menthan-2-one (No. 375) and studies of genotoxicity with dihydrocarveol (No. 378) and carveol (No. 381) were available. These data support the conclusions of the previous evaluation.

### Conclusions

The Committee concluded that the three flavouring agents pinocarvyl isobutyrate (No. 2242), carvyl palmitate (No. 2243) and 6-hydroxycarvone (No. 2244), which are additions to the group of carvone and structurally related substances evaluated previously, do not give rise to safety concerns at current estimated dietary exposures.

The Committee did not re-evaluate (+)-carvone (No. 380.1) and (–)-carvone (No. 380.2) according to the Procedure for the Safety Evaluation of Flavouring Agents given the lack of information on the oral exposure from all sources, the need to review the ADI of (+)-carvone and the lack of toxicological data on (–)-carvone.

For (+)-carvone (No. 380.1), the Committee concluded that a review of the ADI is recommended based on the evaluation of all biochemical and toxicological data. In addition, data are needed for an assessment of oral exposure to (+)-carvone from all sources to complete the re-evaluation. This could not be completed during the current meeting.

For (–)-carvone (No. 380.2), the Committee concluded that additional toxicological data are necessary. Also, data are needed for an assessment of oral exposure to (–)-carvone from all sources to complete the re-evaluation.

The ADI for (+)-carvone is maintained pending review of the ADI at a future meeting. The Committee recommends that the re-evaluation is completed within 3 years.

An addendum to the monograph was prepared.

### References

1. FAO/WHO. Principles and methods for the risk assessment of chemicals in food. A joint publication of the Food and Agriculture Organization of the United Nations and the World Health Organization. Geneva: World Health Organization, International Programme on Chemical Safety; 2009 (Environmental Health Criteria 240).

2. Nijssen LM, van Ingen-Visscher CA, Donders JJH. Volatile compounds in food, version 16.4 [database]. Zeist, the Netherlands: Triskelion B.V.; 2017 (<http://www.vcf-online.nl/VcfHome.cfm>, accessed 15 November 2017).
3. 2015 IOFI Global Poundage Survey. Brussels: International Organization of the Flavor Industry; 2017a. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
4. Interim inquiry on volume use and added use levels for flavoring agents to be presented at the JECFA 86th meeting. Private communication to the International Organization of the Flavor Industry, Brussels; 2017b. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
5. Engel W. Detection of a “nonaromatic” NIH shift during in vivo metabolism of the monoterpene carvone in humans. *J Agric Food Chem*. 2002;50(6):1686–94.
6. Jäger W, Mayer M, Platzer P, Reznicek G, Dietrich H, Buchbauer G. Stereoselective metabolism of the monoterpene carvone by rat and human liver microsomes. *J Pharm Pharmacol*. 2000;52(2):191–7.
7. EFSA. Scientific Opinion on the safety assessment of carvone, considering all sources of exposure. *EFSA J*. 2014;12(7):3806 [74 pp]. doi:10.2903/j.efsa.2014.3806.
8. National Toxicology Program. Toxicology and carcinogenesis studies of *d*-carvone (CAS No. 2244-16-8) in B6C3F1 mice and toxicology studies in F344/N rats (gavage studies). (NTP/TR No. 381, draft; NIH No. 90-2836). Washington, DC, USA: US Department of Health and Human Services; 1989. Draft report submitted to WHO by Flavor and Extract Manufacturer’s Association of the United States, Washington, DC, USA [cited in [Annex 1](#), reference 94].
9. Cramer GM, Ford RA, Hall RL. Estimation of toxic hazard – a decision tree approach. *Food Cosmet Toxicol*. 1978;16(3):255–76.

#### 4.1.3 Furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers

##### Introduction

The Committee re-evaluated 39 flavouring agents belonging to the group of furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers (Nos 1491–1526 and 2103–2105). At three previous meetings, the Committee noted that there were positive in vitro genotoxicity data for several members of this group and that there was a paucity of in vivo genotoxicity data to allay concerns ([Annex 1](#), references 178, 190 and 211). Based on these considerations, the Committee concluded that the Procedure for the Safety Evaluation of Flavouring Agents could not be applied to this group.

The concerns with this group arose primarily from the carcinogenicity of furan itself, which is believed to involve a reactive genotoxic metabolite formed by epoxidation and opening of the furan ring. Furan is not a member of this group of flavouring agents, but all the members of the group contain a furan ring with substituents of varying complexity. Four members of this group, namely 2-methylfuran (No. 1487), 2,5-dimethylfuran (No. 1488), 2-ethylfuran (No. 1489) and 2-butylfuran (No. 1490), have short-chain alkyl substituents on the



furan ring; although considered at previous meetings, these four members are no longer supported by industry and were not considered in this re-evaluation.

At the present meeting, the Committee considered additional studies of *in vitro* genotoxicity (for seven flavouring agents: Nos 1495, 1497, 1503, 1504, 1511, 1514 and 1520) and *in vivo* genotoxicity (for four flavouring agents: Nos 1491, 1497, 1503 and 1511). Additional short-term studies of toxicity for two flavouring agents in this group (Nos 1491 and 1500) were also available.

Twenty of these 39 flavouring agents (Nos 1491–1494, 1497, 1499, 1503–1505, 1508–1513, 1520–1522, 2104 and 2105) have been reported to occur as natural components in foods including cheese, chicken, cocoa, coffee, honey, rye bread, spirituous beverages, tomatoes, wheaten bread, wine and other foods [1].

The re-evaluations of the 39 flavouring agents in this group were conducted using the Procedure for the Safety Evaluation of Flavouring Agents. The first step in the Procedure is consideration of genotoxicity ([Annex 1](#), reference 230).

### Assessment of dietary exposure

The total annual volumes of production of the 39 flavouring agents belonging to the group of furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers is 585 kg in Europe, 798 kg in the USA, 246 kg in Japan and 490 kg in Latin America [2, 3]. More than 83%, 70%, 93% and 85% of the annual production volume in Europe, the USA, Japan and Latin America, respectively, is accounted for by 2-furyl methyl ketone (No. 1503).

Dietary exposures were estimated using both the SPET and the MSDI method, and the higher of the two values for each flavouring agent is reported in [Table 14](#). Estimated daily dietary exposures range from 0.3 to 1200 µg/person (SPET values) and from 0.01 to 61 µg/person (MSDI method values). The estimated daily dietary exposure is highest for 2,5-dimethyl-3-oxo-(2*H*)-furan-4-yl butyrate (No. 1519) at 1200 µg/person, the SPET value for non-alcoholic beverages. The SPET yielded the highest estimated daily dietary exposure in each case with the exception of phenethyl 2-furoate (No. 1517; MSDI = 2 µg/person).

### Absorption, distribution, metabolism and excretion

Detailed information on the ADME of this group of flavouring agents was described in the monograph of the sixty-ninth meeting of the Committee ([Annex 1](#), reference 191). At that meeting, the Committee noted that the biotransformation processes applicable to members of this group of furan-substituted flavouring agents are, in large part, dependent on the presence or absence of specific functional groups attached to the furan ring. The Committee also noted that at higher dose levels, low relative molecular mass alkyl furans (e.g. 2-methylfuran)

can undergo ring oxidation to yield reactive 2-ene-1,4-dicarbonyl intermediates that can react with protein and DNA. For example, furan has limited metabolic options and is biotransformed via ring oxidation to an enedialdehyde species that is a potent hepatotoxin. The Committee further noted that the presence of an extended side-chain attached to the furan ring would reduce the potential for epoxidation of the double bond and provide a site for detoxication via metabolism and elimination.

No new data on the ADME of specific members of this group of flavouring agents were available for the present meeting.

### Consideration of genotoxicity data

Additional studies of genotoxicity on furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers were evaluated for the present meeting. A total of 16 in vitro genotoxicity studies were available for seven flavouring agents (Nos 1495, 1497, 1503, 1504, 1511, 1514 and 1520), and a total of eight in vivo genotoxicity studies were available for four flavouring agents (Nos 1491, 1497, 1503 and 1511).

A positive result was observed for 2-furyl methyl ketone (No. 1503) in an in vitro SCE assay. However, an in vitro chromosomal aberration assay was negative and in vivo studies of DNA damage (comet assays) and micronucleus induction were also negative. All other in vitro and in vivo genotoxicity assays considered at the present meeting were negative.

The Committee concluded that the newly available in vitro and in vivo genotoxicity data evaluated at the present meeting allay the previous concerns of the Committee. Those concerns arose primarily from the carcinogenicity of furan itself and from some positive genotoxicity findings for four flavouring agents with short-chain alkyl substituents on the furan ring. Those four flavouring agents, namely 2-methylfuran (No. 1487), 2,5-dimethylfuran (No. 1488), 2-ethylfuran (No. 1489) and 2-butylfuran (No. 1490), are no longer supported by industry, and were not considered in this re-evaluation.

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

**Step 1.** Genotoxicity data on members of this group of 39 flavouring agents do not raise concerns for genotoxicity.

**Step 2.** In applying the Procedure for the Safety Evaluation of Flavouring Agents, the Committee assigned all 39 flavouring agents to structural class III [4].

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

**Step 4.** The highest estimated dietary exposures for 14 flavouring agents (Nos 1492, 1500, 1502, 1512, 1517, 1518, 1522–1526, 2103–2105) are below the class III threshold of toxicological concern (i.e. 90 µg/day). The Committee

therefore concluded that these 14 flavouring agents would not pose a safety concern at current estimated dietary exposures.

The highest estimated dietary exposures for 25 flavouring agents (Nos 1491, 1493–1499, 1501, 1503–1511, 1513–1516, 1519–1521) are above the class III threshold of toxicological concern. Therefore, these 25 flavouring agents proceeded to Step 5 of the Procedure.

**Step 5.** For 2-pentylfuran (No. 1491) the NOAEL of 30 mg/kg bw per day from a 90-day study in rats [5] provides an adequate MOE of 10 000 in relation to the estimated dietary exposure to No. 1491 when used as a flavouring agent. This NOAEL is also appropriate for assessment of the structurally related flavouring agents 2-decylfuran (No. 1493) and 2,4-difurfurylfuran (No. 1496). This NOAEL provides adequate MOEs of 6000 and 4500 in relation to the estimated dietary exposure to No. 1493 and No. 1496, respectively, when used as flavouring agents.

For 3-(2-furyl)acrolein (No. 1497), the NOAEL of 45 mg/kg bw per day from a 90-day study in rats [6] provides an adequate MOE of 5400 in relation to the estimated dietary exposure to No. 1497 when used as a flavouring agent. This NOAEL is also appropriate for assessment of the structurally related flavouring agents 3-methyl-2-(3-methylbut-2-enyl)-furan (No. 1494), 2-methyl-3(2-furyl)acrolein (No. 1498), 3-(5-methyl-2-furyl)prop-2-enal (No. 1499) and 2-furfurylidene-butyraldehyde (No. 1501) and provides adequate MOEs of 9000, 10 800, 2700 and 10 800 in relation to the estimated dietary exposures to Nos 1494, 1498, 1499 and 1501, respectively, when used as flavouring agents.

For 2,3-dimethylbenzofuran (No. 1495), the NOAEL of 0.6 mg/kg bw per day from a 90-day study in rats [7] provides an adequate MOE of 360 in relation to the estimated dietary exposure to No. 1495 when used as a flavouring agent.

For 3-acetyl-2,5-dimethylfuran (No. 1506), the NOAEL of 10 mg/kg bw per day from a 14-day study in rats [8] provides an adequate MOE of 3000 in relation to the estimated dietary exposure to No. 1506 when used as a flavouring agent. The NOAEL of 10 mg/kg bw per day for No. 1506 is also appropriate for assessment of the structurally related flavouring agents 2-acetyl-5-methylfuran (No. 1504) and 2-acetyl-3,5-dimethylfuran (No. 1505) and provides adequate MOEs of 6000 and 600 in relation to the estimated dietary exposures to 1504 and 1505, respectively, when used as flavouring agents.

For 2-furyl methyl ketone (No. 1503), the NOAEL of 25 mg/kg bw per day obtained from a 90-day study in rats [6] provides an adequate MOE of 4300 in relation to the estimated dietary exposure to No. 1503 when used as a flavouring agent. The NOAEL of 25 mg/kg bw per day for No. 1503 is also appropriate for assessment of the structurally related flavouring agents 2-butyrylfuran (No. 1507), (2-furyl)-2-propanone (No. 1508), 2-pentanoylfuran (No. 1509), furfuryl methyl ether (No. 1520) and ethyl furfuryl ether (No. 1521) and provides adequate MOEs of 2400, 10 000, 1500, 7500 and 12 000, respectively, in relation to the estimated

dietary exposures to Nos 1507, 1508, 1509, 1520 and 1521, respectively, when used as flavouring agents.

For 4-(2-furyl)-3-buten-2-one (No. 1511), the NOAEL of 30 mg/kg bw per day from a 14-day study in rats [9] provides an adequate MOE of 2600 in relation to the estimated dietary exposure to No. 1511 when used as a flavouring agent. The NOAEL of 30 mg/kg bw per day for No. 1511 is also appropriate for assessment of the structurally related flavouring agents 1-(2-furyl)butan-3-one (No. 1510), ethyl 3-(2-furyl)propanoate (No. 1513), isobutyl 3-(2-furan)propionate (No. 1514), isoamyl 3-(2-furan)propionate (No. 1515), isoamyl 3-(2-furan)butyrate (No. 1516) provides adequate MOEs of 4500, 3900, 4500, 4500 and 12 000 in relation to the estimated dietary exposures to Nos 1510, 1513, 1514, 1515 and 1516, respectively, when used as flavouring agents.

For 2,5-dimethyl-3-oxo-(2*H*)-fur-4-yl butyrate (No. 1519), the NOAEL of 200 mg/kg bw per day for the structurally related substance 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (No. 1446) obtained from a 2-year study in rats [10] provides an adequate MOE of 10 000 in relation to the estimated dietary exposure to No. 1519 when used as a flavouring agent.

Based on the adequate margins of exposure for each of the 25 flavouring agents considered at Step 5 of the Procedure, the Committee concluded that these 25 flavouring agents (Nos 1491, 1493–1499, 1501, 1503–1511, 1513–1516, 1519–1521) would not pose a safety concern at current estimated dietary exposures.

### Consideration of combined intakes from use as flavouring agents

Twenty-five of the flavouring agents in this group have MSDI values of less than 0.1 µg/day, and the four highest MSDI values are 3, 6, 13 and 61 µg/day. The Committee considered that combined intakes of members of this group of flavouring agents do not raise safety concerns.

### Consideration of secondary components

Two flavouring agents in this group (Nos 1519 and 1524) have a minimum assay value of less than 95% (see [Annex 3](#)). For No. 1519, the major secondary components are 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (present at 1–3%) and butyric acid (present at 1–3%). The SPET value for No. 1519 is 1200 µg/day and 3% of this value is 36 µg/day, which is below the class III threshold of toxicological concern (90 µg/day). Butyric acid (JECFA flavour No. 87) was evaluated at the forty-ninth meeting, and it was concluded that there were no safety concerns from its use as a flavouring agent ([Annex 1](#), reference 131). The major secondary components of No. 1519 are therefore not considered to present a safety concern at estimated dietary exposures from the use of No. 1519 as a flavouring agent.

For No. 1524, the major secondary component is di-(2-methyl-3-furyl) disulfide (present at 6–7%). The SPET value for No. 1524 is 10 µg/day and 7%

of this value is 0.7 µg/day, which is below the class III threshold of toxicological concern (90 µg/day). The major secondary component of No. 1524 is therefore not considered to present a safety concern at estimated dietary exposures from the use of No. 1524 as a flavouring agent.

## Conclusions

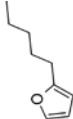
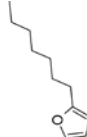
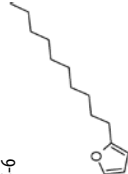
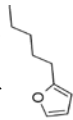
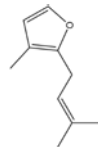


The Committee concluded that the 39 previously evaluated flavouring agents in this group of furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers that were re-evaluated at the present meeting (Nos 1491–1529 and 2103–2105) do not give rise to safety concerns. The Committee concluded that the additional genotoxicity data on members of this group allay the concerns that were raised at previous meetings.

An addendum to the monograph was prepared.

## References

1. Nijssen LM, van Ingen-Visscher CA, Donders JH. Volatile compounds in food, version 16.4 [database]. Zeist, the Netherlands: Triskelion B.V.; 2017 (<http://www.vcf-online.nl/VcfHome.cfm>, accessed 10 February 2018).
2. 2015 IOFI Global Poundage Survey. Brussels: International Organization of the Flavor Industry; 2017a. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
3. Interim inquiry on volume use and added use levels for flavoring agents to be presented at the JECFA 86th meeting. Private communication to the International Organization of the Flavor Industry, Brussels; 2017b. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
4. Cramer GM, Ford RA, Hall RL. Estimation of toxic hazard – a decision tree approach. *Food Cosmet Toxicol.* 1978;16(3):255–76.
5. Bauter M. 2-Pentylfuran: A 90-day oral gavage study in rats. Product Safety Labs, Dayton NJ. Study no. 42348. Unpublished report to the International Organization of the Flavor Industry, 2017. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
6. Lough R. A combined 28-day and 90-day toxicity study of four test articles [2-furyl methyl ketone, benzophenone, 3-(2-furyl)acrolein and isobutyl 3-(2-furyl) propionate] administered orally (in the diet) to the albino rat. Study no. 81238. Unpublished report from Bio-Research Laboratories, Ltd, Montréal, Quebec, Canada; 1985. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
7. Long DW. TT 175: Acute oral toxicity and 3-month oral toxicity study in the rat. Study no. IFREB-R 770261. Unpublished report from Institut Français de Recherches et Essais Biologiques (IFREB), Les Oncins, France; 1977. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
8. Van Miller JP, Weaver EV. Fourteen-day dietary minimum toxicity screen (MTS) of 2-methyl-1-butanol blend, methyl-*o*-methoxy benzoate, 4,5,6,7-tetrahydro-3,6-dimethylbenzofuran, 3-actyl-2,5-dimethylfuran and furfuryl methyl ether in albino rats. Study no. 50-526. Unpublished report from

**Table 14**  
**Summary of the results of the safety evaluations of furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers used as flavouring agents<sup>a,b,c,d</sup>**

Flavouring agent	No.	CAS no. and structure	Step 4 Does the higher of the two predicted dietary exposure estimates exceed the threshold of toxicological concern value for the structural class? <sup>e</sup>	Step 5 Does a NOAEL exist for the flavouring agent or a structural relative that provides an adequate margin of exposure?	Structural relative name (No.) and Structure	Conclusion based on current estimated dietary exposure
<b>Structural class III</b>						
2-Pentylfuran	1491	3777-69-3 	Yes, SPET: 180	Yes. The NOAEL of 30 mg/kg bw per day in a 90-day study in rats [5] is 10 000 times greater than the estimated dietary exposure to No. 1491 when used as a flavouring agent.	NR	No safety concern
2-Heptylfuran	1492	3777-71-7 	No, SPET: 6	NR	NR	No safety concern
2-Decylfuran	1493	83469-85-6 	Yes, SPET: 300	Yes. The NOAEL of 30 mg/kg bw per day in a 90-day study in rats [5] for the related substance 2-pentylfuran (No. 1491) is 6 000 times greater than the estimated dietary exposure to No. 1493 when used as a flavouring agent.	2-Pentylfuran (No. 1491) 	No safety concern
3-Methyl-2-(3-methylbut-2-enyl)-furan	1494	15186-51-3 	Yes, SPET: 300	Yes. The NOAEL of 45 mg/kg bw per day for the related substance 3-(2-furyl)acrolein (No. 1497) [6] is 9 000 times greater than the dietary exposure to No. 1494 when used as a flavouring agent.	3-(2-Furyl)acrolein (No. 1497) 	No safety concern
2,3-Dimethylbenzofuran	1495	3782-00-1 	Yes, SPET: 100	Yes. The NOAEL of 0.6 mg/kg bw per day [7] is 360 times greater than the estimated dietary exposure to No. 1495 when used as a flavouring agent.	NR	No safety concern

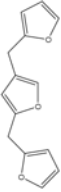
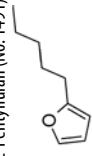

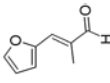

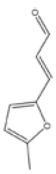

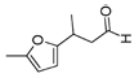
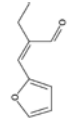

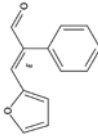
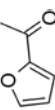
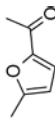

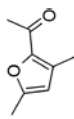

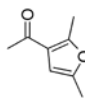
		Step 4 Does the higher of the two predicted dietary exposure estimates exceed the threshold of toxicological concern value for the structural class?*		Step 5 Does a NOAEL exist for the flavouring agent or a structural relative that provides an adequate margin of exposure?		Conclusion based on current estimated dietary exposure	
Flavouring agent	No.	CAS no. and structure	Yes, SPET: 400	Yes, SPET: 500	Yes, The NOAEL of 30 mg/kg bw per day [5] for the related substance 2-pentylfuran (No. 1491) is 4 500 times greater than the estimated dietary exposure to No. 1496 when used as a flavouring agent.	Structural relative name (No.) and Structure	No safety concern
2,4-Difurfurylfuran	1496	64280-32-6 	Yes, SPET: 400			2-Pentylfuran (No. 1491) 	No safety concern
3-(2-Furyl)acrolein	1497	623-30-3 	Yes, SPET: 500		Yes, The NOAEL of 45 mg/kg bw per day [6] is 5 400 times greater than the dietary exposure to No. 1497 when used as a flavouring agent.	NR	No safety concern
2-Methyl-3(2-furyl)acrolein	1498	874-66-8 	Yes, SPET: 250		Yes, The NOAEL of 45 mg/kg bw per day [6] for the related substance 3-(2-furyl)acrolein (No. 1497) is 10 800 times greater than the dietary exposure to No. 1498 when used as a flavouring agent.	3-(2-Furyl)acrolein (No. 1497) 	No safety concern
3-(5-Methyl-2-furyl)prop-2-enal	1499	5555-90-8 	Yes, SPET: 1 000		Yes, The NOAEL of 45 mg/kg bw per day [6] for the related substance 3-(2-furyl)acrolein (No. 1497) is 2 700 times greater than the estimated dietary exposure to No. 1499 when used as a flavouring agent.	3-(2-Furyl)acrolein (No. 1497) 	No safety concern
3-(5-Methyl-2-furyl)butanal	1500	31704-80-0 	No, SPET: 50		NR	NR	No safety concern
2-Furfurylidene-butyraldehyde	1501	770-27-4 	Yes, SPET: 250		Yes, The NOAEL of 45 mg/kg bw per day [6] for the related substance 3-(2-furyl)acrolein (No. 1497) is 10 800 times greater than the estimated dietary exposure to No. 1501 when used as a flavouring agent.	3-(2-Furyl)acrolein (No. 1497) 	No safety concern

Table 14 (continued)

Flavouring agent	No.	CAS no. and structure	Step 4 Does the higher of the two predicted dietary exposure estimates exceed the threshold of toxicological concern value for the structural class?*	Step 5 Does a NOAEL exist for the flavouring agent or a structural relative that provides an adequate margin of exposure?	Structural relative name (No.) and Structure	Conclusion based on current estimated dietary exposure
2-Phenyl-3-(2-furyl)prop-2-enal	1502	65545-81-5 	No, SPET: 40	NR	NR	No safety concern
2-Furyl methyl ketone	1503	1192-62-7 	Yes, SPET: 350	Yes: The NOAEL of 25 mg/kg bw per day [6] is 4 300 times greater than the estimated dietary exposure to No. 1503 when used as a flavouring agent.	NR	No safety concern
2-Acetyl-5-methylfuran	1504	1193-79-9 	Yes, SPET: 100	Yes: The NOAEL of 10 mg/kg bw per day [8] for the related substance 3-acetyl-2,5-dimethylfuran (No. 1506) is 6 000 times greater than the estimated dietary exposure to No. 1504 when used as a flavouring agent.	3-Acetyl-2,5-dimethylfuran (No. 1506) 	No safety concern
2-Acetyl-3,5-dimethylfuran	1505	22940-86-9 	Yes, SPET: 1 000	Yes: The NOAEL of 10 mg/kg bw per day [8] for the related substance 3-acetyl-2,5-dimethylfuran (No. 1506) is 600 times greater than the estimated dietary exposure to No. 1505 when used as a flavouring agent.	3-Acetyl-2,5-dimethylfuran (No. 1506) 	No safety concern
3-Acetyl-2,5-dimethylfuran	1506	10599-70-9 	Yes, SPET: 200	Yes: The NOAEL of 10 mg/kg bw per day [8] is 3 000 times greater than the estimated dietary exposure to No. 1506 when used as a flavouring agent.	NR	No safety concern



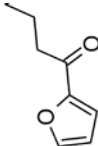
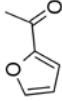
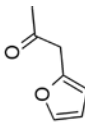
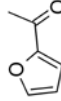
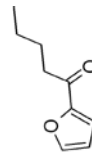
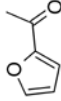
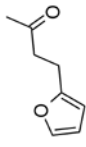
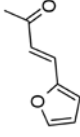
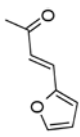
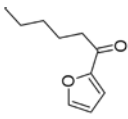
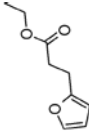
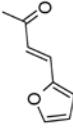
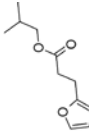
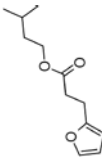
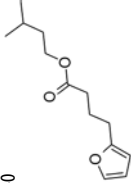
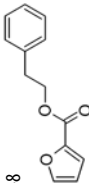
Flavouring agent	No.	CAS no. and structure	Step 4 Does the higher of the two predicted dietary exposure estimates exceed the threshold of toxicological concern value for the structural class?*	Step 5 Does a NOAEL exist for the flavouring agent or a structural relative that provides an adequate margin of exposure?	Structural relative name (No.) and Structure	Conclusion based on current estimated dietary exposure
2-Butyrylfuran	1507	100113-53-9 	Yes, SPET: 625	Yes. The NOAEL of 25 mg/kg bw per day [6] for the related substance 2-furyl methyl ketone (No. 1503) is 2 400 times greater than the estimated dietary exposure to No. 1507 when used as a flavouring agent.	2-Furyl methyl ketone (No. 1503) 	No safety concern
(2-Furyl)-2-propanone	1508	6975-60-6 	Yes, SPET: 150	Yes. The NOAEL of 25 mg/kg bw per day [6] for the related substance 2-furyl methyl ketone (No. 1503) is 10 000 times greater than the estimated dietary exposure to No. 1508 when used as a flavouring agent.	2-Furyl methyl ketone (No. 1503) 	No safety concern
2-Pentanoylfuran	1509	3194-17-0 	Yes, SPET: 1 000	Yes. The NOAEL of 25 mg/kg bw per day [6] for the related substance 2-furyl methyl ketone (No. 1503) is 1 500 times greater than the estimated dietary exposure to No. 1509 when used as a flavouring agent.	2-Furyl methyl ketone (No. 1503) 	No safety concern
1-(2-Furyl)butan-3-one	1510	699-17-2 	Yes, SPET: 400	Yes. The NOAEL of 30 mg/kg bw per day [9] for the related substance 4-(2-furyl)-3-buten-2-one (No. 1511) is 4 500 times greater than the estimated dietary exposure to No. 1510 when used as a flavouring agent.	4-(2-Furyl)-3-buten-2-one (No. 1511) 	No safety concern
4-(2-Furyl)-3-buten-2-one	1511	623-15-4 	Yes, SPET: 699	Yes. The NOAEL of 30 mg/kg bw per day [9] is 2 600 times greater than the estimated dietary exposure to No. 1511 when used as a flavouring agent.	NR	No safety concern
Pentyl 2-furyl ketone	1512	14360-50-0 	No, SPET: 15	NR	NR	No safety concern

Table 14 (continued)

Flavouring agent		Step 4 Does the higher of the two predicted dietary exposure estimates exceed the threshold of toxicological concern value for the structural class?*		Step 5 Does a NOAEL exist for the flavouring agent or a structural relative that provides an adequate margin of exposure?		Conclusion based on current estimated dietary exposure	
No.	CAS no. and structure	Yes, SPET: 463	Yes, SPET: 400	Yes, The NOAEL of 30 mg/kg bw per day [9] for the related substance 4-(2-furyl)-3-buten-2-one (No. 1511) is 3 900 times greater than the estimated dietary exposure to No. 1513 when used as a flavouring agent.	4-(2-Furyl)-3-buten-2-one (No. 1511)	No safety concern	
Ethyl 3-(2-furyl)propanoate	1513 10031-90-0 	Yes, SPET: 463		Yes, The NOAEL of 30 mg/kg bw per day [9] for the related substance 4-(2-furyl)-3-buten-2-one (No. 1511) is 3 900 times greater than the estimated dietary exposure to No. 1513 when used as a flavouring agent.		No safety concern	
Isobutyl 3-(2-furan)propanoate	1514 105-01-1 	Yes, SPET: 400		Yes, The NOAEL of 30 mg/kg bw per day [9] for the related substance 4-(2-furyl)-3-buten-2-one (No. 1511) is 4 500 times greater than the estimated dietary exposure to No. 1514 when used as a flavouring agent.	4-(2-Furyl)-3-buten-2-one (No. 1511)	No safety concern	
Isoamyl 3-(2-furan)propanoate	1515 7779-67-1 	Yes, SPET: 400		Yes, The NOAEL of 30 mg/kg bw per day [9] for the related substance 4-(2-furyl)-3-buten-2-one (No. 1511) is 4 500 times greater than the estimated dietary exposure to No. 1515 when used as a flavouring agent.	4-(2-Furyl)-3-buten-2-one (No. 1511)	No safety concern	
Isoamyl 3-(2-furan)butyrate	1516 7779-66-0 	Yes, SPET: 150		Yes, The NOAEL of 30 mg/kg bw per day [9] for the related substance 4-(2-furyl)-3-buten-2-one (No. 1511) is 12 000 times greater than the estimated dietary exposure to No. 1516 when used as a flavouring agent.	4-(2-Furyl)-3-buten-2-one (No. 1511)	No safety concern	
Phenethyl 2-furoate	1517 7149-32-8 	No, MSDI: 2		NR	NR	No safety concern	

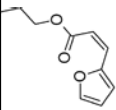
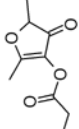

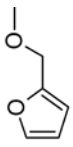
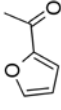
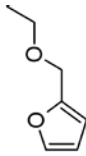
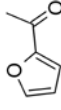
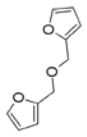
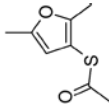
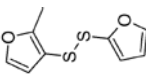
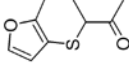
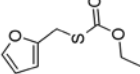
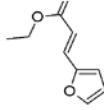

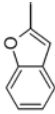
Flavouring agent	No.	CAS no. and structure	Step 4 Does the higher of the two predicted dietary exposure estimates exceed the threshold of toxicological concern value for the structural class? <sup>a</sup>	Step 5 Does a NOAEL exist for the flavouring agent or a structural relative that provides an adequate margin of exposure?	Structural relative name (No.) and Structure	Conclusion based on current estimated dietary exposure
Propyl 2-furanacrylate	1518	623-22-3 		NR	NR	No safety concern
2,5-Dimethyl-3-oxo-(2 <i>H</i> )-furan-4-yl butyrate	1519	114099-96-6 	Yes, SPET: 1 200	Yes. The NOAEL of 200 mg/kg bw per day [10] for the related substance 2,5-dimethyl-4-hydroxy-3(2 <i>H</i> )-furanone (No. 1446) is 10 000 times greater than the estimated dietary exposure to No. 1519 when used as a flavouring agent.	2,5-Dimethyl-4-hydroxy-3(2 <i>H</i> )-furanone (No. 1446) 	No safety concern
Furfuryl methyl ether	1520	13679-46-4 	Yes, SPET: 200	Yes. The NOAEL of 25 mg/kg bw per day [6] for the related substance 2-furyl methyl ketone (No. 1503) is 7 500 times greater than the estimated dietary exposure to No. 1520 when used as a flavouring agent.	2-Furyl methyl ketone (No. 1503) 	No safety concern
Ethyl furfuryl ether	1521	6270-56-0 	Yes, SPET: 125	Yes. The NOAEL of 25 mg/kg bw per day [6] for the related substance 2-furyl methyl ketone (No. 1503) is 12 000 times greater than the estimated dietary exposure to No. 1521 when used as a flavouring agent.	2-Furyl methyl ketone (No. 1503) 	No safety concern
Difurfuryl ether	1522	4437-22-3 	No, SPET: 6	NR	NR	No safety concern
2,5-Dimethyl-3-furanthiol acetate	1523	55764-22-2 	No, SPET: 2	NR	NR	No safety concern

Table 14 (continued)

Flavouring agent	No.	CAS no. and structure	Step 4 Does the higher of the two predicted dietary exposure estimates exceed the threshold of toxicological concern value for the structural class? <sup>a</sup>	Step 5 Does a NOAEL exist for the flavouring agent or a structural relative that provides an adequate margin of exposure?	Structural relative name (No.) and Structure	Conclusion based on current estimated dietary exposure
Furfuryl(2-methyl-3-furyl)disulfide	1524	109537-55-5 	No, SPET: 10	NR	NR	No safety concern
3-[(2-Methyl-3-furyl)thio]-2-butanone	1525	61295-44-1 	No, SPET: 0.3	NR	NR	No safety concern
O-Ethyl S-(2-furylmethyl)thiocarbonate	1526	376595-42-5 	No, SPET: 9	NR	NR	No safety concern
(E)-Ethyl 3-(2-furyl)acrylate	2103	53282-12-5 	No, SPET: 20	NR	NR	No safety concern

Flavouring agent	No.	CAS no. and structure	Chemical structure	Step 4 Does the higher of the two predicted dietary exposure estimates exceed the threshold of toxicological concern value for the structural class? <sup>a</sup>	Step 5 Does a NOAEL exist for the flavouring agent or a structural relative that provides an adequate margin of exposure?	Structural relative name (No.) and Structure	Conclusion based on current estimated dietary exposure
Di-2-furylmethane	2104	1197-40-6		No, SPET: 40	NR	NR	No safety concern
2-Methylbenzofuran	2105	4265-25-2		No, SPET: 3	NR	NR	No safety concern

bw: body weight; CAS: Chemical Abstracts Service; MSDI: maximized survey-derived intake; no.: number; NOAEL: no-observed-adverse-effect level; NR: not required; SPET: single-portion exposure technique

<sup>a</sup> The Committee re-evaluated 39 flavouring agents belonging to the group of furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides and ethers. All 39 flavouring agents have been considered previously by the Committee.

<sup>b</sup> Step 1: Genotoxicity data on the flavouring agents in this group do not raise concerns for genotoxicity.

<sup>c</sup> Step 2: All 39 flavouring agents belong to structural class III.

<sup>d</sup> Step 3: Dietary exposures were estimated using both the SPET and the MSDI method, and the higher of the two values for each flavouring agent is reported. Dietary exposure values are expressed in µg/day.

<sup>e</sup> Step 4: The threshold for human dietary exposure for structural class III is 90 µg/day.

Bushy Run Research Center, Export, Pennsylvania, USA; 1987. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.

9. Gill MW, Van Miller JP. Fourteen-day dietary minimum toxicity screen (MTS) in albino rats. Study no. 50-528. Unpublished report from Bushy Run Research Center, Export, Pennsylvania, USA to the Flavor and Extract Manufacturers Association, Washington, DC, USA; 1987. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
10. Kelly CM, Bolte HF. ST 07 C99: A 24-month dietary carcinogenicity study in rats. Unpublished report no. 99-2644. Huntingdon Life Sciences, East Millstone, NJ, USA to the Flavor and Extract Manufacturers Association, Washington, DC, USA; 2003. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.

#### 4.1.4 Linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters

##### Introduction

The Committee evaluated two additional flavouring agents belonging to the group of linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters. One of the flavouring agents was a linear unsaturated aldehyde, *trans*-6-octenal (No. 2240), and the other was a branched unsaturated alcohol, 2,6-dimethyl-5-heptenol (No. 2241). The evaluations were conducted using the Procedure for the Safety Evaluation of Flavouring Agents ([Annex 1](#), reference 230).

The Committee evaluated 42 other members of this group of flavouring agents at its fifty-first meeting ([Annex 1](#), reference 137). For 41 of the 42 substances in this group, the Committee concluded that there were no safety concerns at estimated dietary exposures. The evaluation of the remaining substance, ethyl 2-methyl-3,4-pentadienoate (No. 353), was completed at the sixty-eighth meeting ([Annex 1](#), reference 187). The Committee evaluated 20 other members of this group of flavouring agents at its sixty-first meeting ([Annex 1](#), reference 166). The Committee concluded that there were no safety concerns at estimated dietary exposures with respect to all of these 20 flavouring agents. The Committee evaluated nine other members of this group of flavouring agents at its seventy-sixth meeting ([Annex 1](#), reference 211). The Committee concluded that all nine flavouring agents were of no safety concern at estimated dietary exposures.

Both of the additional flavouring agents (Nos 2240 and 2241) in this group have been reported to occur naturally and can be found in ginger [1].

A comprehensive literature search was conducted in PubMed; no additional relevant studies were identified.

### Assessment of dietary exposure

The total annual volume of production of the two additional flavouring agents is 0.2 kg [2, 3]. The volume of the annual production in the USA is completely accounted for by *trans*-6-octenal (No. 2240). In Japan, the production volume is completely accounted for by 2,6-dimethyl-5-heptenol (No. 2241).

Dietary exposures were estimated using both the SPET and the MSDI method, with the highest values reported in Table 15. The higher estimated daily dietary exposure is for 2,6-dimethyl-5-heptenol (No. 2241), at 300 µg/day, the SPET value obtained from nonalcoholic soft beverages. For *trans*-6-octenal (No. 2240), the estimates of daily dietary exposures via the MSDI method and SPET are 0.03 and 40 µg/day, respectively.

### Absorption, distribution, metabolism and excretion

Information on the ADME of the flavouring agents belonging to the group of linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters has been described in the monographs of the fifty-first, sixty-first, sixty-eighth, and seventy-sixth meetings (Annex 1, references 138, 167, 188, 212).

The aliphatic esters are expected to hydrolyse to their analogous unsaturated aliphatic alcohol and carboxylic acids during passage through the gastrointestinal tract [4, 5, 6]. The resulting linear and branched-chain unsaturated primary alcohols are expected to be absorbed and further oxidized to their analogous aldehydes and acids, and rapidly absorbed [7, 8]. The absorbed aldehydes are oxidized to their analogous unsaturated carboxylic acids. These unsaturated carboxylic acids undergo further enzymatic conversion prior to entry into the  $\beta$ -oxidation pathway, where they are fully metabolized to carbon dioxide and water via the citric acid cycle.

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

**Step 1.** There are no adequate chemical-specific genotoxicity studies on the additional flavouring agents. However, there are no structural alerts for these compounds. Data from related flavouring agents indicate that these do not show genotoxic potential based on uniformly negative genotoxicity data.

**Step 2.** In applying the Procedure for the Safety Evaluation of Flavouring Agents to *trans*-6-octenal (No. 2240) and 2,6-dimethyl-5-heptenol (No. 2241), the Committee assigned both flavouring agents to structural class I [9].

**Steps 3 and 4.** Dietary exposures using both the MSDI method and SPET have been determined. The highest estimated dietary exposures of both flavouring agents in structural class I were below the threshold of concern (i.e. 1800 µg/person per day for class I). The Committee therefore concluded that both

flavouring agents (Nos 2240–2241) would not pose a safety concern at current estimated dietary exposures.

### Consideration of combined intakes from use as flavouring agents

The two additional flavouring agents in this group of linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters have low MSDIs (0.01–0.03 µg/day). The Committee concluded that consideration of combined intakes is not necessary, because the additional flavouring agents would not contribute significantly to the combined intake of this flavouring group.

### Consideration of secondary components

One flavouring agent in this group, 2,6-dimethyl-5-heptenol (No. 2241), has a minimum assay value of less than 95% (see [Annex 3](#)). The major secondary component, 2,6-dimethyl-5-heptenal (No. 349), present at 1–6%, does not present a safety concern at estimated dietary exposures from the use of No. 2241 as a flavouring agent.

### Consideration of additional data on previously evaluated flavouring agents

In the previous evaluation of substances in this group of linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters, studies of biochemistry, acute toxicity, short-term and long-term toxicity and genotoxicity were available ([Annex 1](#), references 137, 166, 187 and 211). None of the 71 flavouring agents in this group raised safety concerns.

No adequate studies on the additional flavouring agents were available.

For previously evaluated flavouring agents in this group, studies of acute toxicity (Nos 336, 1269, 1286 and 1640), studies of short-term toxicity (Nos 330, 332 and 333) and studies of genotoxicity (Nos 315, 329, 330, 333, 334, 346, 349, 1272, 1286 and 1637) were available. The studies available for the present evaluation support the conclusions drawn by previous safety evaluations.


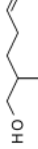
### Conclusions

The Committee concluded that the two flavouring agents *trans*-6-octenal (No. 2240) and 2,6-dimethyl-5-heptenol (No. 2241), which are additions to the group of linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters evaluated previously, do not give rise to any safety concerns at current estimated dietary exposures.

An addendum to the monograph was prepared.



Table 15  
**Summary of the results of the safety evaluations of two additional flavouring agents in the group of linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters<sup>a,b</sup>**

Flavouring agent	No.	CAS no. and structure	Step 4 <sup>c</sup> Does the highest dietary exposure estimate exceed the threshold of concern?	Step 5 Does a NOAEL exist for the flavouring agent or a structural relative that provides an adequate margin of exposure?	Conclusion based on current estimated dietary exposure
<b>Structural class I</b>					
<i>trans</i> -6-Octenal	2240	63196-63-4 	No, SPET: 40	NR	No safety concern
2,6-Dimethyl-5-heptenal	2241	4234-93-9 	No, SPET: 300	NR	No safety concern

CAS: Chemical Abstracts Service; NOAEL: no-observed-adverse-effect level; NR: not relevant; SPET: single-portion exposure technique

<sup>a</sup> Seventy-one flavouring agents in this group were previously evaluated by the Committee (Annex 1, References 137, 166, 187 and 211).

<sup>b</sup> Step 2. Two flavouring agents (Nos. 2240–2241) are in structural class I.

<sup>c</sup> The threshold for human dietary exposure for structural class I is 1800 µg/day. All dietary exposure values are expressed in µg/day. The dietary exposure value listed represents the highest estimated dietary exposure, which was calculated using the SPET.

## References

1. Nijssen LM, van Ingen-Visscher CA, Donders JH. Volatile compounds in food, version 16.4: [database]. Zeist, the Netherlands: Triskelion B.V.; 2017 (<http://www.vcf-online.nl/VcfHome.cfm>, accessed 15 November 2017).
2. 2015 IOFI Global Poundage Survey. International Organization of the Flavor Industry, Brussels; 2017a. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
3. Interim inquiry on volume use and added use levels for flavoring agents to be presented at the JECFA 86th meeting. Private communication to the International Organization of the Flavor Industry, Brussels; 2017b. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
4. Gangolli SD, Shilling WH. Hydrolysis of esters by artificial gastric and pancreatic juices. Study no. 11/1968. Unpublished report to the Flavor and Extracts Manufacturers Association, Washington, DC, USA; 1968. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
5. Junge W, Heymann E. Characterization of the isoenzymes of pig-liver esterase: 2. Kinetic studies. *Eur J Biochem.* 1979;95(3):519–25.
6. Heymann E. Carboxylesterases and amidases. In: W. Jakoby, editor. *Enzymatic basis of detoxication*. New York: Academic Press; 1980.
7. Dawson AM, Holdsworth CD, Webb J. Absorption of short chain fatty acids in man. *Proc Soc Exp Biol Med.* 1964;117:97–100.
8. Gaillard D, Derache R. [Metabolism of different alcohols present in alcoholic beverages, in the rat]. *Trav Soc Pharm Montp.* 1965;25:51–62.
9. Cramer GM, Ford RA, Hall RL. Estimation of toxic hazard – a decision tree approach. *Food Cosmet Toxicol.* 1978;16(3):255–76.

### 4.1.5 Maltol and related substances

#### Introduction

The Committee evaluated two flavouring agents belonging to the group of maltol and related substances: one additional flavouring agent, ethyl maltol isobutyrate (No. 2252), and one flavouring agent, maltol (No. 1480) that was being re-evaluated. The evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents ([Annex 1](#), reference 230). Ethyl maltol isobutyrate (No. 2252) has not been previously evaluated by the Committee. Maltol (No. 1480) was re-evaluated because new *in vitro* and *in vivo* genotoxicity data had become available.

The Committee previously evaluated seven members of this group of flavouring agents at its sixty-fifth meeting ([Annex 1](#), reference 178). The Committee concluded that all seven flavouring agents were of no safety concern at estimated dietary exposures and maintained the previously established ADIs of 0–1 mg/kg bw for maltol (No. 1480) and 0–2 mg/kg bw for ethyl maltol (No. 2252).

Maltol (No. 1480) was evaluated at the eleventh meeting ([Annex 1](#), reference 14), when a temporary ADI of 0–1 mg/kg bw was established because no long-term studies of toxicity were available. At the eighteenth meeting ([Annex 1](#), reference 35), the Committee withdrew the temporary ADI because the results of long-term studies of toxicity requested at the previous ADI meeting had not been made available. At the twenty-second meeting ([Annex 1](#), reference 47), the Committee evaluated new data on toxicity and established a temporary ADI of 0–0.5 mg/kg bw. At its twenty-fifth meeting, the Committee evaluated additional data and established an ADI of 0–1 mg/kg bw for maltol on the basis of a NOEL of 100 mg/kg bw in rats ([Annex 1](#), reference 56).

Ethyl maltol (No. 1481) was evaluated at the fourteenth meeting ([Annex 1](#), reference 22), when the Committee established an ADI of 0–2 mg/kg bw. At its eighteenth meeting ([Annex 1](#), reference 35), the Committee re-evaluated ethyl maltol and re-affirmed the ADI of 0–2 mg/kg bw.

Maltol (No. 1480) has been reported to occur naturally in a wide variety of foods such as wheat and rye breads, milk, butter, uncured pork, beer, cocoa, coffee, peanuts, soy proteins, beans and clams [1]. During baking (for example, of bread or beans) and roasting (for example, of cocoa, coffee, peanuts), simple sugars are partly converted to maltol [1, 2].

A comprehensive literature search was performed in Scopus; two additional relevant studies were identified.

### Assessment of dietary exposure

The total annual volume of production of the additional flavouring agent (ethyl maltol isobutyrate, No. 2252) is 29 kg in Japan [3, 4]. No production volume was reported for Europe, Latin America or the USA.

The total annual volume of production for the flavouring agent presented for re-evaluation, maltol (No. 1480), is approximately 83 200 kg in Europe, 87 600 kg in the USA, 12 500 kg in Japan and 47 600 kg in Latin America [3, 4].

Dietary exposure was estimated using both the SPET and the MSDI method, with the highest values reported in [Table 16](#). The estimated daily dietary exposure is highest for maltol (10 440 µg/day, SPET value for nonalcoholic “soft” beverages). The MSDI values for the four regions range from 2625 to 9091 µg/day. For ethyl maltol isobutyrate (No. 2252), the estimated daily dietary exposures were 8 µg/day (MSDI value) and 400 µg/day (SPET value).

### Absorption, distribution, metabolism and excretion

Information on the ADME of flavouring agents belonging to the group of maltol and related substances is described in the monograph of the sixty-fifth meeting ([Annex 1](#), reference 179).

Chemically, maltol is classified as a  $\gamma$ -pyrone. It is a hydroxyl-substituted 4*H*-pyran-4-one and is anticipated to be metabolized like phenol, which primarily undergoes phase II conjugation of the free hydroxyl substituent. Maltol and ethyl maltol are predominantly metabolized to sulfate and glucuronic acid conjugates, which are then eliminated in the urine [5]. Ethyl maltol isobutyrate (No. 2252) is predicted to be hydrolysed to ethyl maltol and the corresponding simple aliphatic carboxylic acid (isobutyric acid) [6].

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

**Step 1.** There are no structural alerts for genotoxicity for maltol (No. 1480) or ethyl maltol isobutyrate (No. 2252). Chemical-specific genotoxicity data available for maltol (No. 1480) do not indicate that this flavouring agent has genotoxic potential.

**Step 2.** In applying the Procedure for the Safety Evaluation of Flavouring Agents, the Committee assigned one flavouring agent (No. 1480) to structural class II and one flavouring agent (No. 2252) to structural class III [7].

**Step 3.** The highest dietary exposures were estimated using the SPET for both flavouring agents.


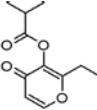

**Step 4.** The highest estimated dietary exposure for maltol (No. 1480) is above the threshold of toxicological concern (i.e. 540  $\mu\text{g}/\text{day}$  for class II). Accordingly, the evaluation of this flavouring agent proceeded to Step 5 of the Procedure.

The highest estimated dietary exposure for ethyl maltol isobutyrate (No. 2252) is above the threshold of toxicological concern (i.e. 90  $\mu\text{g}/\text{day}$  for class III). Accordingly, the evaluation of this flavouring agent proceeded to Step 5 of the Procedure.

**Step 5.** For maltol, the NOAEL of 125 mg/kg bw per day from a 90-day study in dogs [8] provides an MOE of 720 in relation to the estimated daily dietary exposure to No. 1480 (SPET = 10 440  $\mu\text{g}/\text{day}$  or 174  $\mu\text{g}/\text{kg}$  bw per day) when used as a flavouring agent. The Committee therefore concluded that maltol (No. 1480) would not pose a safety concern at current estimated dietary exposures.

At its fourteenth meeting ([Annex 1](#), reference 22), the Committee established an ADI of 0–2 mg/kg bw for the structurally related substance ethyl maltol (No. 1481) on the basis of a NOEL of 200 mg/kg bw per day in a 2-year dietary study in rats [8]. This ADI was maintained at the sixty-fifth meeting ([Annex 1](#), reference 178). The NOEL of 200 mg/kg bw per day provides an adequate MOE of 28 570 in relation to estimated daily dietary exposure to No. 2252 (SPET = 400  $\mu\text{g}/\text{day}$  or 7  $\mu\text{g}/\text{kg}$  bw per day) when used as a flavouring agent. The Committee concluded that ethyl maltol isobutyrate (No. 2252) would not pose a safety concern at current estimated dietary exposures.

Table 16  
**Summary of the results of the safety evaluations of maltol and related substances used as flavouring agents<sup>a,b</sup>**

Flavouring agent	No.	CAS no. and structure	Step 4 <sup>c</sup>		Step 5 <sup>d</sup>		Conclusion based on current estimated dietary exposure
			Does the highest dietary exposure estimate exceed the threshold of toxicological concern?	Does a NOAEL exist for the flavouring agent or a structurally related substance that provides an adequate margin of exposure?	Related structure name (No.) and structure		
<b>Structural class I</b>							
Maltol <sup>e</sup>	1480	118-71-8 	Yes, SPEI: 10 440	Yes. The NOAEL of 125 mg/kg bw per day from a 90-day study in dogs (8) is 720 times the estimated dietary exposure to No. 1480 when used as a flavouring agent.			No safety concern
<b>Structural class III</b>							
Ethyl maltol isobutyrate	2252	852997-28-5 	Yes, SPEI: 400	Yes. The NOEL of 200 mg/kg bw per day in a 2-year dietary study in rats for the structurally related substance ethyl maltol (No. 1481) (8) is 28 570 times the estimated dietary exposure to No. 2252 when used as a flavouring agent.	Ethyl maltol <sup>f</sup> (No. 1481) 		No safety concern

bw: body weight; CAS: Chemical Abstracts Service; no.: number; NOAEL: no-observed-adverse-effect level; NOEL: no-observed-effect level; NR: not relevant; SPEI: single-portion exposure technique

<sup>a</sup> Seven flavouring agents in this group were previously evaluated by the Committee (Annex 1, reference 179).

<sup>b</sup> Step 2. One flavouring agent is in structural class II and one flavouring agent is in structural class III.

<sup>c</sup> The threshold of toxicological concern for human dietary exposure for structural class II is 540 µg/day and for structural class III is 90 µg/day. All dietary exposure values are expressed in µg/day. The dietary exposure values listed represent the highest estimated daily dietary exposures calculated using the SPEI.

<sup>d</sup> The margin of exposure was calculated based on the highest estimated daily dietary exposure calculated using the SPEI.

<sup>e</sup> The previously established ADI for maltol was withdrawn by the Committee.

<sup>f</sup> The previously established ADI for ethyl maltol of 0–2 mg/kg bw was maintained.

Table 16 summarizes the evaluations of the two flavouring agents (Nos 1480 and 2252) belonging to this group of maltol and related substances.

### Consideration of combined intakes from use as flavouring agents

The additional flavouring agent, ethyl maltol isobutyrate (No. 2252), in the group of maltol and related substances has a low MSDI (8 µg/day). The Committee concluded that consideration of combined intakes is not necessary because the additional flavouring agent would not contribute significantly to the combined intake of this flavouring group.

### Consideration of secondary components

Ethyl maltol isobutyrate (No. 2252) has a minimum assay of less than 95% (see Annex 3). The major secondary component in No. 2252, present at 2–3%, is ethyl maltol (No. 1481). This flavouring agent had been previously evaluated by the Committee (Annex 1, reference 178) and did not pose a safety concern at the estimated dietary exposure.

### Consideration of additional data on previously evaluated flavouring agents

For two previously evaluated flavouring agents in this group, maltol (No. 1480) and maltyl isobutyrate (No. 1482), additional studies of acute toxicity (No. 1482) and genotoxicity (Nos 1480 and 1482) were available for the present evaluation. These additional data raised no safety concerns and support the previous safety evaluations.

However, the Committee could not verify the NOEL of 100 mg/kg bw in rats that was used to derive the ADI of 0–1 mg/kg bw for maltol at its twenty-fifth meeting because of uncertainties in the administered dose levels and the effects observed in several studies as described in the monograph of that meeting (Annex 1, reference 56). The Committee noted that a NOAEL of 125 mg/kg bw per day was identified in a published 90-day study in dogs [8]. This NOAEL was used to complete the re-evaluation of maltol as a flavouring agent.

### Conclusions

In the previous evaluation of flavouring agents in this group of maltol and related substances, biochemical data and studies of acute toxicity, short-term toxicity, long-term toxicity and carcinogenicity, genotoxicity and reproductive toxicity were available. None of the seven previously evaluated flavouring agents raised safety concerns based on the estimated dietary exposures and the biochemical and toxicological data available (Annex 1, reference 179).

The Committee concluded that the two flavouring agents (Nos. 2252 and 1480) under evaluation, one of which is an addition to the group of maltol

and related substances evaluated previously, do not give rise to safety concerns at current estimated dietary exposures.

However, the Committee could not verify the NOEL of 100 mg/kg bw in rats that was used to derive the ADI of 0–1 mg/kg bw for maltol during its twenty-fifth meeting because of uncertainties in the administered dose levels and the effects observed in several studies described in the monograph of that meeting (Annex 1, reference 57). The Committee concluded that access to either the original studies or submission of new data would be needed to reaffirm or amend the current ADI. The Committee therefore withdrew the ADI for maltol pending review of the appropriate data at a future meeting. The ADI for ethyl maltol was maintained.

An addendum to the monograph was prepared.

## References

1. Nijssen LM, van Ingen-Visscher CA, Donders JH. Volatile compounds in food, version 16.4 [database]. Zeist, the Netherlands: TNO Triskelion B.V.; 2017 (<http://www.vcf-online.nl/VcfHome.cfm>, accessed 15 November 2017).
2. Belitz H-D, Grosch W, Schieberle P. Food Chemistry, 4th ed. Berlin Heidelberg, Germany: Springer-Verlag; 2009.
3. 2015 IOFI Global Poundage Survey. International Organization of the Flavor Industry, Brussels; 2017a. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
4. Interim inquiry on volume use and added use levels for flavoring agents to be presented at the JECFA 86th meeting. Private communication to the International Organization of the Flavor Industry, Brussels; 2017b. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
5. Rennhard HH. The metabolism of ethyl maltol and maltol in the dog. *J Agric Food Chem.* 1971;19(1):152–4.
6. Bennett C. Hydrolysis of maltol propionate via pancreatic secretion. Private communication to the International Organization of the Flavor Industry, Brussels; 1998. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
7. Cramer GM, Ford RA, Hall RL. Estimation of toxic hazard – a decision tree approach. *Food Cosmet Toxicol.* 1978;16(3):255–76.
8. Gralla EJ, Stebbins RB, Coleman GL, Delahunt CS. Toxicity studies with ethyl maltol. *Toxicol Appl Pharmacol.* 1969;15(3):604–13.

### 4.1.6 Menthol and structurally related substances

#### Introduction

The Committee evaluated seven flavouring agents belonging to the group of menthol and structurally related substances, which was previously evaluated. The Committee re-evaluated menthol (No. 427) and evaluated six additional flavouring agents. These included four menthyl esters (menthyl formate [No. 2246], menthyl propionate [No. 2247], *l*-menthyl butyrate [No. 2248] and

dimenthyl glutarate [No. 2250]), *dl*-isomenthol (No. 2249) and one polyether alcohol, ( $\pm$ )-2-[(2-*p*-menthoxy)ethoxy]ethanol (No. 2251). The evaluations were conducted using the Procedure for the Safety Evaluation of Flavouring Agents ([Annex 1](#), reference 230).

The Committee previously evaluated menthol and 13 other members of this group of flavouring agents at its fifty-first meeting ([Annex 1](#), references 137) and 10 additional members of the group at its sixty-ninth meeting ([Annex 1](#), reference 190). The Committee concluded that all 24 flavouring agents were of no safety concern at estimated dietary exposures.

Menthol (No. 427) was first evaluated by the Committee at the eleventh meeting when an unconditional ADI of 0–0.2 mg/kg bw and a conditional<sup>4</sup> ADI of 0.2–2 mg/kg bw was established ([Annex 1](#), reference 14). At the eighteenth meeting, an ADI of 0–0.2 mg/kg bw was established for menthol ([Annex 1](#), reference 35). This ADI was maintained at the twentieth meeting. At the twentieth meeting, the Committee requested additional long-term studies of toxicity and carcinogenicity in rats, information on the average and likely maximum dietary exposure to menthol, clinical observations of humans with higher than average dietary exposure to menthol and studies of metabolism ([Annex 1](#), reference 41). At the fifty-first meeting, an ADI of 0–4 mg/kg bw was established on the basis of the NOEL of 380 mg/kg bw per day in a long-term study in rats, applying a safety factor of 100 and rounding to one significant figure ([Annex 1](#), reference 137).

Menthol (No. 427), menthyl formate (No. 2246) and *dl*-isomenthol (No. 2249) have been reported to occur naturally in foods and can be mainly found in peppermint oil and other *Mentha* species oils. Menthol (10–70%) and menthone (No. 429; 7–40%) are the principal constituents of peppermint oil [2, [Annex 1](#), reference 137].

Menthol (No. 427) was re-evaluated because new data had become available since the previous evaluation. Menthol and the other flavourings were evaluated at the request of the Forty-eighth Session of the CCFA [3].

A comprehensive literature search was performed in Scopus; one additional reference was identified.

### Assessment of dietary exposure

The total annual volume of production for menthol (No. 427) is 296 000 kg in Europe, 496 000 kg in the USA, 146 000 kg in Japan and 127 000 kg in Latin America [4, 5].

<sup>4</sup> "Conditional ADI is a term no longer used by JECFA to signify a range above the 'unconditional ADI', which may signify an acceptable intake when special problems, different patterns of dietary intake, and special groups of the population that may require consideration are taken into account." [1]



The total annual volume of production of the six additional flavouring agents in the group of menthol and structurally related substances is 14 900 kg in Europe, 103 937 kg in the USA and 92 kg in Japan. No production volume was reported for Latin America [4, 5].

Of the seven flavouring agents under evaluation by this current Committee, menthol (No. 427) accounts for more than 95% of the total annual production volume in Europe and 82% in the USA. Dimethyl glutarate (No. 2250) accounts for almost all the remaining volume. Menthol (No. 427) accounts for more than 99% of the annual production volume in Japan and 100% in Latin America.

Dietary exposures were estimated using both the SPET and the MSDI method, with the highest values reported in Table 17. The highest estimated dietary exposure is for menthol (No. 427; 51 474 µg/day, MSDI value; the SPET value is 42 210 µg/day). For the other flavouring agents, the estimated daily dietary exposures range from 0.03 to 10 784 µg/day (MSDI values) and from 300 to 9000 µg/day (SPET values), with the SPET yielding the highest estimate in all but one case (dimethyl glutarate [No. 2250]).

### Absorption, distribution, metabolism and excretion

Information on the ADME of the flavouring agents belonging to the group of menthol and structurally related substances has previously been described in the monographs of the eleventh, twentieth and fifty-first meetings (Annex 1, references 15, 42 and 138). Additional information was available for the current meeting.

The menthyl esters in this group (Nos 2246–2248 and 2250) can be expected to be readily hydrolysed to menthol and their respective carboxylic acids [6, 7]. Similar to menthol, *dl*-isomenthol (No. 2249) is expected to be conjugated with glucuronic acid and be eliminated in the urine or faeces [8, 9, 10]. The polyether alcohol conjugate of menthol, (±)-2-[(2-*p*-menthoxy)ethoxy] ethanol (No. 2251) is also expected to undergo conjugation with glucuronic acid and subsequent elimination in the urine and faeces.

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

**Step 1.** There are no structural alerts for genotoxicity for these flavouring agents. Chemical-specific genotoxicity data available for menthol (No. 427), *dl*-isomenthol (No. 2249) and (±)-2-[(2-*p*-menthoxy)ethoxy] ethanol (No. 2251) indicate that these flavouring agents are unlikely to be genotoxic based on the weight of evidence.

**Step 2.** In applying the Procedure for the Safety Evaluation of Flavouring Agents, the Committee assigned six flavouring agents (Nos 427, 2246–2250) to structural class I and one flavouring agent (No. 2251) to structural class III [11].

**Step 3.** The highest dietary exposures were estimated using the SPET for five of the seven flavouring agents (Nos 2246–2249, 2251) and the MSDI method for menthol (No. 427) and dimethyl glutarate (Nos 2250).

**Step 4.** The highest estimated dietary exposures for three of the six flavouring agents in structural class I (Nos 2246–2248) are below the threshold of toxicological concern (i.e. 1800 µg/day for class I). The Committee therefore concluded that these flavouring agents would not pose a safety concern at current estimated dietary exposures.

The highest estimated dietary exposures for the remaining three flavouring agents in structural class I (Nos 427, 2249 and 2250) are above the threshold of toxicological concern. Therefore, the evaluation of these flavouring agents proceeded to Step 5.

The highest estimated dietary exposure for the one flavouring agent in structural class III (No. 2251) is above the threshold of toxicological concern (i.e. 90 µg/day for class III). Therefore, the evaluation of this flavouring agent proceeded to Step 5.

**Step 5.** For menthol (No. 427), an ADI of 0–4 mg/kg bw was allocated on the basis of the NOEL of 380 mg/kg bw per day from a 2-year dietary study in rats, applying a safety factor of 100 and rounding to one significant figure (Annex 1, reference 137). The NOEL of 380 mg/kg bw per day from a 2-year dietary study in rats [12] provides an adequate MOE of 440 in relation to the estimated dietary exposure to No. 427 (MSDI of 51 474 µg/day). The Committee therefore concluded that menthol (No. 427) would not pose a safety concern when used as a flavouring agent at current estimated dietary exposures.

For *dl*-isomenthol (No. 2249), the NOEL of 380 mg/kg bw per day from a 2-year dietary study in rats [12] of the structurally related substance menthol (No. 427) provides an adequate MOE of 7600 in relation to the estimated dietary exposure to No. 2249 (SPET value of 3000 µg/day). The Committee therefore concluded that *dl*-isomenthol (No. 2249) would not pose a safety concern when used as a flavouring agent at current estimated dietary exposures.

For dimethyl glutarate (No. 2250), the NOEL of 380 mg/kg bw per day from a 2-year dietary study in rats [12] for the structurally related substance menthol (No. 427) provides an adequate MOE of 2100 in relation to the estimated dietary exposure to No. 2250 (MSDI value of 10 784 µg/day). The Committee therefore concluded that dimethyl glutarate (No. 2250) would not pose a safety concern when used as a flavouring agent at current estimated dietary exposures.

For (±)-2-[(2-*p*-methoxy)ethoxy]ethanol (No. 2251), the NOEL of 30 mg/kg bw per day from a 90-day study in rats for the structurally related substance 3-*l*-menthoxypropane-1,2-diol (No. 1408) [13] provides an adequate MOE of 1700 in relation to the estimated dietary exposure to No. 2251 (SPET value of 1000 µg/day). The Committee therefore concluded that (±)-2-[(2-*p*-methoxy)

ethoxy]ethanol (No. 2251) would not pose a safety concern when used as a flavouring agent at current estimated dietary exposures.

Table 17 summarizes the evaluations of the seven flavouring agents belonging to this group of menthol and structurally related substances (Nos 427 and 2246–2251).

### Consideration of combined intakes from use as flavouring agents

With the exception of dimethyl glutarate (No. 2250), all additional flavouring agents to the group of menthol and structurally related substances have low MSDI values (range: 0.03–21 µg/day). The highest MSDI value for dimethyl glutarate (No. 2250) is 10 784 µg/day. No production volume for No. 2250 was reported for Japan and Latin America. The Committee concluded that consideration of combined intakes is not necessary for Japan and Latin America because the additional flavouring agents would not contribute significantly to the combined intake of this flavouring group.

In the unlikely event that the flavouring agents with the common metabolite menthol (No. 427) in this group were to be consumed together with menthol (No. 427) on a daily basis, the estimated combined intakes<sup>5</sup> for the four flavouring agents (Nos 427, 429, 1414 and 2250) with the highest estimated dietary exposures (MSDI values) would be 24 622 µg/day in Europe and 65 627 µg/day in the USA. The estimated combined intake would therefore exceed the human threshold of toxicological concern (1800 µg/day for structural class I). However, the vast majority of the combined intake would be due to menthol alone, for which an ADI of 0–4 mg/kg bw was previously established. The estimated combined intake does not exceed this ADI, which is equal to 240 mg/day for a 60 kg person. Also, as the flavouring agents are likely to be metabolized efficiently, they would not saturate metabolic pathways.

Therefore, the Committee concluded that combined intake would not raise safety concerns.

### Consideration of additional data on previously evaluated flavouring agents




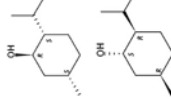
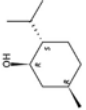
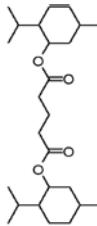
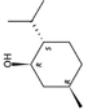
For eight previously evaluated flavouring agents in this group, additional studies of metabolism (No. 429); studies of acute toxicity (Nos 430, 1854 and 1856); and studies of genotoxicity (Nos 429, 430, 431, 445, 1856 and 1857) were available.

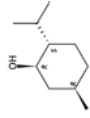
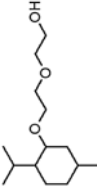
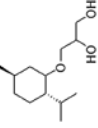
### Conclusions

In the previous evaluations of substances in this group of menthol and structurally related substances, biochemical data; studies of acute toxicity, short-term toxicity,

<sup>5</sup> Combined intake was calculated on a molar basis relative to the formation of a common metabolite. In this case, the common metabolite is menthol, with a relative molecular mass of 157.

Table 17  
**Summary of the results of safety evaluations of menthol and six additional structurally related substances used as flavouring agents<sup>a,b</sup>**

Flavouring agent	No.	CAS no. and structure	Step 4 <sup>c</sup>	Step 5 <sup>d</sup>	Related structure name (No.) and structure	Conclusion based on current estimated dietary exposure
			Does the highest dietary exposure estimate exceed the threshold of toxicological concern?	Does a NOAEL exist for the flavouring agent or a structurally related substance that provides an adequate margin of exposure?		
<b>Structural class I</b>						
Menthyl formate	2246	2230-90-2 	No, SPEI: 400	NR	NR	No safety concern
Menthyl propionate	2247	86014-82-6 	No, SPEI: 600	NR	NR	No safety concern
<i>l</i> -Menthyl butyrate	2248	68366-64-3 	No, SPEI: 300	NR	NR	No safety concern
<i>dl</i> -Isomenthol	2249	3623-52-7 	Yes, SPEI: 3 000	Yes. The NOEL of 380 mg/kg bw per day from a 2-year dietary study in rats for the structurally related substance menthol [12] is 7 600 times the estimated dietary exposure to No. 2249 when used as a flavouring agent.	Menthol (No. 427) 	No safety concern
Dimenthyl glutarate	2250	406179-71-3 	Yes, MSDI: 10 784	Yes. The NOEL of 380 mg/kg bw per day from a 2-year dietary study in rats for the structurally related substance menthol [12] is 2 100 times the estimated dietary exposure to No. 2250 when used as a flavouring agent.	Menthol (No. 427) 	No safety concern

Flavouring agent	No.	CAS no. and structure	Step 4 <sup>a</sup>		Step 5 <sup>d</sup>		Conclusion based on current estimated dietary exposure
			Does the highest dietary exposure estimate exceed the threshold of toxicological concern?	Does a NOAEL exist for the flavouring agent or a structurally related substance that provides an adequate margin of exposure?	Related structure name (No.) and structure	Conduction based on current estimated dietary exposure	
Menthol <sup>b</sup>	427	89-78-1 	Yes; MSDI: 51 474	Yes. The NOEL of 380 mg/kg bw per day from a 2-year dietary study in rats [12] is 440 times the estimated dietary exposure to No. 427 when used as a flavouring agent.	NR	No safety concern	
<b>Structural class III</b>							
(±)-2-(2- <i>p</i> -Methoxyethoxy)ethanol	2251	28804-53-7 	Yes; SPEI: 1 000	Yes. The NOEL of 30 mg/kg bw per day from a 90-day study in rats for the structurally related substance 3- <i>l</i> -menthoxypropane-1,2-diol [13] is 1 700 times the estimated dietary exposure to No. 2251 when used as a flavouring agent.	3- <i>l</i> -menthoxypropane-1,2-diol (No. 1408) 	No safety concern	

bw: body weight; CAS: Chemical Abstracts Service; MSDI: maximized survey-derived intake; NOAEL: no-observed-adverse-effect-level; NOEL: no-observed-effect level; no.: number; NR: not relevant; SPEI: single-portion exposure technique

<sup>a</sup> Twenty-four flavouring agents in this group were previously evaluated by the Committee (Annex 1, references 137 and 190).

<sup>b</sup> Step 2: Six flavouring agents (Nos 427, 2246–2250) are in structural class I and one flavouring agent (No. 2251) is in structural class III.

<sup>c</sup> The thresholds for toxicological concern for structural class I and structural class III are 1800 µg/day and 90 µg/day, respectively. All dietary exposure values are expressed in µg/day. The dietary exposure values listed represent the highest daily dietary exposure calculated using either the SPEI or the MSDI method. The SPEI gave the highest estimated dietary exposure in each case except for Nos 427 and 2250 where the MSDI value was higher.

<sup>d</sup> The margin of exposure was calculated based on the highest estimated daily dietary exposure using either the SPEI or the MSDI method.

<sup>e</sup> The acceptable daily intake of menthol of 0–4 mg/kg bw established at the fifty-first meeting (Annex 1, reference 137) was maintained.

long-term toxicity and carcinogenicity, genotoxicity, developmental toxicity, immunotoxicity and sensitivity; and human data were available. None of the 24 previously evaluated flavouring agents raised safety concerns based on the estimated dietary exposures and the biochemical and toxicological data available (Annex 1, references 138 and 191).

For the flavouring agent under re-evaluation, menthol (No. 427), additional biochemical data, studies of acute toxicity and genotoxicity and a case study were available. Studies of genotoxicity were available for the *dl*-isomenthol (No. 2249) and ( $\pm$ )-2-[(2-*p*-menthoxy)ethoxy]ethanol (No. 2251).

The studies available for the present evaluation raised no safety concerns and support the previous safety evaluations. The additional data on menthol did not indicate a need to revise the ADI of menthol (No. 427).

The Committee concluded that the seven flavouring agents under evaluation, six of which are additions to the group of menthol and structurally related substances evaluated previously, do not give rise to safety concerns at current estimated dietary exposures. The previously established ADI of 0–4 mg/kg bw for menthol was maintained.

An addendum to the monograph was prepared.

## References

1. Principles and methods for the risk assessment of chemicals in food. A joint publication of the Food and Agriculture Organization of the United Nations and the World Health Organization. Geneva: World Health Organization, International Programme on Chemical Safety; 2009 (Environmental Health Criteria 240).
2. Nijssen LM, van Ingen-Visscher CA, Donders JH. Volatile compounds in food, version 16.4 [database]. Zeist, the Netherlands: TNO Triskelion B.V.; 2017 (<http://www.vcf-online.nl/VcfHome.cfm>, accessed 15 November 2017).
3. 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; and Geneva: World; 2016 (REP16/FA).
4. 2015 IOFI Global Poundage Survey. International Organization of the Flavor Industry, Brussels; 2017a. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
5. Interim inquiry on volume use and added use levels for flavoring agents to be presented at the JECFA 86th meeting. Private communication to the International Organization of the Flavor Industry, Brussels; 2017b. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
6. Heymann E. Carboxylesterases and amidases. In: Jakoby WB, editor, Enzymatic basis of detoxification. New York, NY, USA: Academic Press; 1980.
7. Anders MW. Biotransformation and bioactivation of xenobiotics by the kidney. In: Hutson DH, Caldwell J, Paulson GD, editors. Intermediary xenobiotic metabolism in animals. New York, NY, USA: Taylor and Francis; 1989.

8. Williams RT. Studies in detoxication: The biological reduction of *l*-menthone to *d*-neomenthol and of *d*-isomenthone to *d*-isomenthol in the rabbit. The conjugation of *d*-neomenthol with glucuronic acid. *Biochem J.* 1940;34:690–7.
9. Atzl G, Bertel M, Daxenbichler G, Gleispach H. Determination of etheral oils from the urine by gas-liquid chromatography. *Chromatographia.* 1972;5(4):250–5.
10. Yamaguchi T, Caldwell J, Farmer PB. Metabolic fate of [*3H*]-*l*-menthol in the rat. *Drug Metab Dispos.* 1994;22(4):616–24.
11. Cramer GM, Ford RA, Hall RL. Estimation of toxic hazard – a decision tree approach. *Food Cosmet Toxicol.* 1978;16(3):255–76.
12. Bioassay of *DL*-menthol for possible carcinogenicity. US National Cancer Institute Report NCI-CG-TR-98. US National Technical Information Services Report No. PB-288761, Bethesda, Maryland, USA; 1979 [cited in [Annex I](#), reference 138].
13. Wolfe GW. 91-Day subchronic oral toxicity study in rats with D1156.02 (Laboratory project identification HWA, study no. 297-648). Unpublished report from Hazelton Washington, Inc., Vienna, Virginia, USA; 1992. Submitted to WHO by the Flavor and Extract Manufacturers' Association of the United States [cited in [Annex I](#), reference 174].

#### 4.1.7 Miscellaneous nitrogen-containing substances

##### Introduction

The Committee evaluated three additional flavouring agents belonging to the group of miscellaneous nitrogen-containing substances, which was evaluated previously. The additional flavouring agents included a triazole moiety with a thiopyridine side-chain, 2-(((3-(2,3-dimethoxyphenyl)-1*H*-1,2,4-triazol-5-yl)thio)methyl)pyridine (No. 2235); a benzothiadiazine moiety with a piperidinyl side-chain, (S)-1-(3-(((4-Amino-2,2-dioxido-1*H*-benzo[*c*][1,2,6]thiadiazin-5-yl)oxy)methyl)piperidin-1-yl)-3-methylbutan-1-one (No. 2236); and an *N*-pyrazole- and *N*-thiophene-substituted amide, 2-(4-methylphenoxy)-*N*-(1*H*-pyrazol-3-yl)-*N*-(thiophen-2-ylmethyl)acetamide (No. 2237). The evaluations were conducted using the Procedure for the Safety Evaluation of Flavouring Agents ([Annex 1](#), reference 230).

The Committee considered whether (S)-1-(3-(((4-amino-2,2-dioxido-1*H*-benzo[*c*][1,2,6]thiadiazin-5-yl)oxy)methyl)piperidin-1-yl)-3-methylbutan-1-one (No. 2236) belonged to the group of aliphatic and aromatic amines and amides. This group includes the structurally related 3[(4-amino-2,2-dioxido-1*H*-2,1,3-benzothiadiazin-5-yl)oxy]-2,2-dimethyl-*N*-propylpropanamide (No. 2082), which was evaluated at the seventy-sixth meeting ([Annex 1](#), reference 211). The Committee concluded that the additional flavouring agent should remain in this group. All three flavouring agents that were evaluated at this meeting are reported to be flavour modifiers.

The Committee previously evaluated 16 additional members of this group of miscellaneous nitrogen-containing substances at its sixty-fifth meeting

(Annex 1, reference 178); 14 additional members at its sixty-ninth meeting (Annex 1, reference 190); two additional members at its seventy-sixth meeting (Annex 1, reference 211); and two additional members at its seventy-ninth meeting (Annex 1, reference 220). At each meeting, the Committee concluded that all of the evaluated flavouring agents were of no safety concern at estimated dietary exposures.

None of the additional flavouring agents (Nos 2235, 2236 and 2237) in this group have been reported to occur naturally.

A comprehensive literature search on data on the additional flavouring agents was conducted; no additional studies were identified.

### Assessment of dietary exposure

The total annual volume of production of the three flavouring agents belonging to the group of miscellaneous nitrogen-containing substances is 0.3 kg in the USA. No data on intake were reported for Europe, Japan or Latin America [1, 2]. Each of these flavouring agents contributes equally to the annual production volume in the USA.

Dietary exposures were estimated using both SPET and the MSDI method. The highest values are reported in Table 18. The estimated daily dietary exposure is highest for 2-(((3-(2,3-dimethoxyphenyl)-1*H*-1,2,4-triazol-5-yl)thio)methyl)pyridine (No. 2235), at 800 µg/person per day, the SPET value obtained from soups and broths. For each of the three flavouring agents the estimate from the MSDI method was 0.01 µg/day. For Nos 2236 and 2237 the highest SPET estimates were 750 and 600 µg/person per day, respectively.

### Absorption, distribution, metabolism and excretion

Information on the ADME of the flavouring agents belonging to this group has previously been described in the monographs of the sixty-fifth, sixty-ninth, seventy-sixth and seventy-ninth meetings (Annex 1, references 179, 191, 212 and 221). Additional information was available for this meeting.

Metabolic studies show that Nos 2236 and 2237 have low bioavailability [3, 4]. The data for No. 2235 showed that it was bioavailable with a short half-life in rat plasma. No. 2235 was shown to be metabolized in toxicokinetic and in vivo metabolism studies in the rat [5]. All these additional flavouring agents undergo limited hydrolysis, sulfoxidation or oxidation of the heterocyclic rings or are expected to be excreted unchanged in the faeces or urine [3, 4, 5].

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

**Step 1.** There are no structural alerts for genotoxicity for the three additional flavouring agents (Nos 2235–2237). Chemical-specific genotoxicity data available



for each of these flavouring agents indicate that they lack the potential to be genotoxic.

**Step 2.** All three flavouring agents (Nos 2235–2237) were assigned to structural class III [6].

**Step 3.** The highest dietary exposures were estimated using SPET (Table 18).

**Step 4.** The highest dietary exposure estimates of the three flavouring agents in structural class III were above the threshold of toxicological concern (i.e. 90 µg/person per day for class III). These flavouring agents proceeded to Step 5.

**Step 5.** For these flavouring agents, the NOAELs of 100 mg/kg bw per day in rats, the highest dose tested in 90-day oral toxicity studies [3, 5], provide adequate margins of exposure (7500, 8000 and 10 000, respectively) relative to the highest estimated dietary exposure to No. 2235 (SPET = 800 µg/day), No. 2236 (SPET = 750 µg/day) and No. 2237 (SPET = 600 µg/day) when used as flavouring agents. The Committee therefore concluded that Nos 2235–2237 would not pose safety concerns at current estimated dietary exposures.

Table 18 summarizes the evaluations of the three flavouring agents belonging to this group of miscellaneous nitrogen-containing substances (Nos 2235–2237).

### Consideration of combined intakes from use as flavouring agents

The three additional flavouring agents in this group of miscellaneous nitrogen-containing substances have low MSDIs (0.01 µg/day). The Committee concluded that consideration of combined intakes is not necessary because the additional flavouring agents would not contribute significantly to the combined intake of this flavouring group.

### Consideration of additional data on previously evaluated flavouring agents

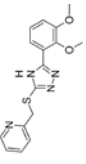
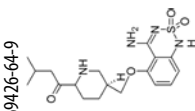
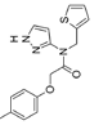
For previously evaluated flavouring agents in this group, studies of acute toxicity (No. 1566 and 1889), genotoxicity (No. 1566) and reproductive/developmental toxicity (No 2161) were available.

### Conclusions

In previous evaluations of flavouring agents in this group of miscellaneous nitrogen-containing substances, biochemical data; studies of acute toxicity, short-term toxicity, long-term toxicity and carcinogenicity, genotoxicity and developmental and reproductive toxicity; and thyroid toxicity were available. The estimated dietary exposures and the biochemical and toxicological data available did not raise any safety concerns for the 34 previously evaluated flavouring agents in this group (Annex 1, references 178, 190, 211 and 220).

For the additional flavouring agents in this group, biochemical data (on Nos 2235–2237); studies of short-term toxicity (on Nos 2235–2237); studies on

Table 18  
**Summary of the results of the safety evaluations of miscellaneous nitrogen-containing substances<sup>a,b</sup>**

Flavouring agent	No.	CAS no. and structure	Step 4 <sup>c</sup> Does the highest dietary exposure estimate exceed the threshold of toxicological concern?	Step 5 <sup>d</sup> Does a NOAEL exist for the flavouring agent or a structurally related substance that provides an adequate margin of exposure?	Conclusion based on current estimated dietary exposure
<b>Structural class III</b>					
2-(((3-(2,3-Dimethoxyphenyl)-1 <i>H</i> -1,2,4-triazol-5-yl)thio)methyl)pyridine	2235	902136-79-2 	Yes, SPEI: 800	Yes, The NOAEL of 100 mg/kg bw per day [5] is 7 500 times the estimated dietary exposure to No. 2235 when used as a flavouring agent.	No safety concern
( <i>S</i> )-1-(3-(((4-Amino-2,2-dioxido-1 <i>H</i> -benzotriazol-1,2,6-thiadiazin-5-yl)oxy)methyl)piperidin-1-yl)-3-methylbutan-1-one	2236	1469426-64-9 	Yes, SPEI: 750	Yes, The NOAEL of 100 mg/kg bw per day [3] is 8 000 times the estimated dietary exposure to No. 2236 when used as a flavouring agent.	No safety concern
2-(4-Methylphenoxy)- <i>N</i> -(1 <i>H</i> -pyrazol-3-yl)- <i>N</i> -(thiophen-2-yl)methyl)acetamide	2237	1374760-95-8 	Yes, SPEI: 600	Yes, The NOAEL of 100 mg/kg bw per day [4] is 10 000 times the estimated dietary exposure to No. 2237 when used as a flavouring agent.	No safety concern

bw: body weight; CAS: Chemical Abstracts Service; NOAEL: no-observed-adverse-effect level; SPEI: single-portion exposure technique

<sup>a</sup> Thirty-four flavouring agents in this group were previously evaluated by the Committee (Annex 1, references 178, 190, 211 and 220).

<sup>b</sup> Step 2: All three flavouring agents are in structural class III.

<sup>c</sup> The threshold for toxicological concern for structural class III is 90 µg/day. All dietary exposure values are expressed in µg/day. The dietary exposure value listed represents the highest estimated dietary exposure calculated using the SPET.

<sup>d</sup> The margin of exposure was calculated based on estimated dietary exposure calculated by the SPET.

genotoxicity (on Nos 2235–2237 and a metabolite of 2237); and developmental studies (on Nos 2236 and 2237) were available.

The studies available on the previously evaluated and additional flavouring agents in this group available for the present evaluation raised no safety concerns and support the previous safety evaluations.

The Committee concluded that these three flavouring agents, which are additions to the group of miscellaneous nitrogen-containing substances evaluated previously, would not give rise to safety concerns at current estimated dietary exposures.

An addendum to the monograph was prepared.

## References

1. 2015 IOFI Global Poundage Survey. International Organization of the Flavor Industry, Brussels; 2017a. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
2. Interim inquiry on volume use and added use levels for flavoring agents to be presented at the JECFA 86th meeting. Private communication to the International Organization of the Flavor Industry, Brussels; 2017b. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
3. Arthur AJ, Karanewsky DS, Luksic M, Goodfellow G, Daniels J. Toxicological evaluation of two flavors with modifying properties: 3-((4-amino-2,2-dioxido-1*H*-benzo[*c*][1,2,6]thiadiazin-5-yl)oxy)-2,2-dimethyl-*N*-propylpropanamide and (S)-1-(3-(((4-amino-2,2-dioxido-1*H*-benzo[*c*][1,2,6]thiadiazin-5-yl)oxy)methyl)piperidin-1-yl)-3-methylbutan-1-one. *Food Chem Toxicol.* 2015;76:33–45.
4. Karanewsky DS, Arthur AJ, Liu H, Chi B, Markison S. Toxicological evaluation of a novel cooling compound: 2-(4-methylphenoxy)-*N*-(1*H*-pyrazol-3-yl)-*N*-(2-thienylmethyl)acetamide. *Toxicol Rep.* 2015;2:1291–309.
5. Karanewsky DS, Arthur AJ, Liu H, Chi B, Ida L, Markison S. Toxicological evaluation of a novel umami flavour compound: 2-(((3-(2,3-dimethoxyphenyl)-1*H*-1,2,4-triazol-5-yl)thio)methyl)pyridine. *Toxicol Rep.* 2016;3:501–12.
6. Cramer GM, Ford RA, Hall RL. Estimation of toxic hazard – a decision tree approach. *Food Cosmet Toxicol.* 1978;16(3):255–76.

### 4.1.8 Saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids

The Committee evaluated two additional flavouring agents belonging to the group of saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids that was evaluated previously. The additional flavouring agents were 8-methyldecanal (No. 2238) and 8-methylnonanal (No. 2239). The evaluations were conducted using the Procedure for the Safety Evaluation of Flavouring Agents ([Annex 1](#), reference 230). Neither of these substances had been previously evaluated.

The Committee evaluated 25 members of this group at its forty-ninth meeting ([Annex 1](#), reference 131). The Committee concluded that all 25 flavouring

agents did not raise any safety concerns at estimated dietary exposures. At its seventy-sixth meeting, the Committee evaluated four additional members of this group of flavouring agents and concluded that all four were of no safety concern at estimated dietary exposures ([Annex 1](#), reference 211).

8-Methylnonanal (No. 2239) was reported to occur naturally in citrus fruits. 8-Methyldecanal (No. 2238) is not reported to occur naturally [1].

A comprehensive literature search was conducted in PubMed; no additional relevant studies were identified.

### Assessment of dietary exposure

The total annual volume of production of the two flavouring agents belonging to the group of saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids is 0.1 kg in the USA, accounted for by 8-methyldecanal (No. 2238), and 0.1 kg in Japan, accounted for by 8-methylnonanal (No. 2239) [2, 3].

Dietary exposures were estimated using both the SPET and the MSDI method, with highest values reported in [Table 19](#). The highest estimated daily dietary exposure for 8-methyldecanal (No. 2238) is 0.1 µg/day (SPET value). The highest estimated daily dietary exposure of 8-methylnonanal (No. 2239) is 0.03 µg/day (MSDI value).

### Absorption, distribution, metabolism and excretion

Information on the ADME of the flavouring agents belonging to the group of saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids has previously been described in the monograph of the forty-ninth meeting ([Annex 1](#), references 132). Additional information on the ADME of saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids was available for this meeting. The previously described information as well as the additional information available for this meeting, on the ADME of the flavouring agents belonging to this group, are summarized as follows.

The substances in this group share common metabolic pathways, and are expected to be absorbed into the gastrointestinal tract [4, 5]. Shorter branched-chain aliphatic alcohols, aldehydes and acids undergo β-oxidation cleavage, with intermediates metabolized to CO<sub>2</sub> via the tricarboxylic acid cycle. Longer alkyl chain length and branching increases susceptibility to both oxidation and glucuronidation in aliphatic alcohols, as there is an increase in the affinity for UDP-glucuronosyltransferases [6, 7]. Longer and more substituted alcohols produce polar metabolites after undergoing ω-, ω-1 and β-oxidation and selective dehydrogenation and hydration, resulting in chain-shortening [8]. The substances in this group are expected to be metabolized to innocuous products via common metabolic pathways, or be excreted in the urine.

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

**Step 1.** There are neither structural alerts for genotoxicity nor chemical-specific genotoxicity data on the additional flavouring agents. New and previously evaluated data from other related flavouring agents indicate that these flavouring agents are not likely to be genotoxic. Therefore, the weight of evidence indicates that these additional saturated aliphatic acyclic branched-chain primary aldehyde flavouring agents are not likely to be genotoxic.

**Step 2.** In applying the Procedure for the Safety Evaluation of Flavouring Agents to the above-mentioned flavouring agents, the Committee assigned both flavouring agents to structural class I [9].

**Steps 3 and 4.** Dietary exposures using both MSDI method and SPET have been determined. The highest estimated dietary exposures of both flavouring agents in structural class I were below the threshold of concern (i.e. 1800 µg/person per day for Class I). The Committee therefore concluded that both flavouring agents (Nos 2238 and 2239) would not pose a safety concern at current estimated dietary exposures.

## Consideration of combined intakes from use as flavouring agents

The two additional flavouring agents in this group of saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids have low MSDIs (0.01–0.03 µg/day). The Committee concluded that consideration of combined intakes is not necessary, because the additional flavouring agents would not contribute significantly to the exposure to this flavouring group.



## Consideration of additional data on previously evaluated flavouring agents

In the previous evaluation of substances in this group of saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids, studies of biochemistry, acute toxicity, short-term and long-term toxicity, reproductive and developmental toxicity and genotoxicity were available ([Annex 1](#), references 132 and 211). None of the 29 flavouring agents of this group raised safety concerns.

For the present evaluation, no relevant studies were available for the two additional flavouring agents (Nos 2238 and 2239). For previously evaluated flavouring agents in this group, studies of acute toxicity (Nos 251, 252, 253, 254, 258, 260, 267, 268, 269 and 2176), studies of short-term toxicity (Nos 251, 252, 254, 258, 267, 269, 272 and 275), studies of long-term toxicity (No. 252), studies of genotoxicity (Nos 52, 251, 252, 253, 255, 259, 268, 270, 272 and 275), studies of reproductive and developmental toxicity (Nos 251, 252, 267, 268 and 272) and neurotoxicity (No. 251) were available.

There are positive genotoxicity data, not previously evaluated, for isobutyraldehyde (No. 252) in an in vitro and in vivo chromosomal aberration assay, an in vitro SCE assay and an in vitro forward mutation assay; and for

**Table 19**  
**Summary of the results of the safety evaluations of saturated aliphatic branched-chain primary alcohols, aldehydes and acids used as flavouring agents<sup>a,b</sup>**

Flavouring agent	No.	CAS no. and structure	Step 4 <sup>c</sup> Does the highest dietary exposure estimate exceed the threshold of toxicological concern?	Step 5 Does a NOAEL exist for the flavouring agent or a structurally related substance that provides an adequate margin of exposure?	Conclusion based on current estimated dietary exposure
<b>Structural class I</b>					
8-Methyldecanal	2238	127793-88-8 	No, SPET: 0.1	NR	No safety concern
8-Methylnonanal	2239	3085-26-5 	No, MSDI: 0.03	NR	No safety concern

CAS: Chemical Abstracts Service; MSDI: maximized survey-derived intake; no.: number; NOAEL: no-observed-adverse-effect level; NR: not required; SPET: single-portion exposure technique

<sup>a</sup> Twenty-nine flavouring agents in this group were previously evaluated by the Committee (Annex 1, references 132 and 211).

<sup>b</sup> Step 2: Both flavouring agents are in structural class I.

<sup>c</sup> The threshold for toxicological concern for structural class I is 1800 µg/day, respectively. All dietary exposure values are expressed in µg per day. The dietary exposure value listed represents the highest daily per capita intake calculated either by SPET or MSDI. The SPET gave the highest estimated dietary exposure for No. 2238 and the MSDI gave the highest estimated dietary exposure for No. 2239.

isobutyric acid (No. 253) in an in vitro forward mutation assay. The bacterial reverse mutation assays, and the in vivo micronucleus assays on isobutyraldehyde (No. 252), were negative. The in vivo chromosomal aberration assay using isobutyraldehyde (No. 252) was only positive at the highest dose, which produced notable signs of cytotoxicity. The negative in vivo results for isobutyraldehyde (No. 252) are consistent with the 2-year inhalational carcinogenicity mouse and rat assays that showed nasal toxicity but no carcinogenicity [10, 11]. Therefore, the weight of evidence indicates that these saturated aldehyde flavouring agents are not likely to be genotoxic. The results of the remainder of the genotoxicity assays on flavouring agents in this group are negative.

The studies available for the present evaluation support the previous safety evaluations.

## Conclusions

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

An addendum to the monograph was prepared.

## References

1. Nijssen LM, van Ingen-Visscher CA, Donders JJH. Volatile compounds in food, version 16.3 [database]. Zeist, the Netherlands: TNO Triskelion B.V.; 2017 (<http://www.vcf-online.nl/VcfHome.cfm>).
2. 2015 IOFI Global Poundage Survey. International Organization of the Flavor Industry, Brussels; 2017a. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
3. Interim inquiry on volume use and added use levels for flavoring agents to be presented at the JECFA 86th meeting. Private communication to the International Organization of the Flavor Industry, Brussels; 2017b. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
4. Dawson AM, Holdsworth CD, Webb J. Absorption of short chain fatty acids in man. *Proc Soc Exp Biol Med.* 1964;117:97–100.
5. Gaillard D, Derache R. [Metabolism of different alcohols present in alcoholic beverages, in the rat.] *Trav Soc Pharm Montp.* 1965;25:51–62.
6. Iwersen S, Schmoldt A. ADH-independent metabolism of aliphatic alcohols: Comparison of oxidation and glucuronidation. *Adv Forensic Sci.* 1995;4:19–22.
7. Jurowich S, Sticht G, Käferstein H. Glucuronidation of aliphatic alcohols in human liver microsomes in vitro. *Alcohol.* 2004;32:187–94.
8. Veenstra G, Webb C, Sanderson H, Belanger SE, Fisk P, Nielsen A et al. Human health risk assessment of long chain alcohols. *Ecotoxicol Environ Saf.* 2009;72(4):1016–30.
9. Cramer GM, Ford RA, Hall RL. Estimation of toxic hazard – a decision tree approach. *Food Cosmet Toxicol.* 1978;16(3):255–76.

10. Abdo KM, Haseman JK, Nyska A. Isobutyraldehyde administered by inhalation (whole body exposure) for up to thirteen weeks or two years was a respiratory tract toxicant but was not carcinogenic in F344/N rats and B6C3F1 mice. *Toxicol Sci.* 1998;42(2):136–51.
11. NTP toxicology and carcinogenesis studies of isobutyraldehyde (CAS No. 78-84-2) in F344/N rats and B6C3F1 mice (inhalation studies). Research Triangle Park, North Carolina, USA: US Department of Health and Human Services, National Toxicology Program; 1999 (NTP Technical Report 472).

## 4.2 Specifications of identity and purity of flavouring agents

### 4.2.1 New and maintained specifications

The Committee received information related to specifications for 20 of the 21 new flavouring agents from the call for data for the present meeting. While carvone (Flavor and Extract Manufacturers Association of the United States [FEMA] No. 2249) was listed in the call for data as a new flavouring agent, the Committee noted that full specifications already existed for (+)-carvone (No. 380.1; *d*-carvone) and (–)-carvone (No. 380.2; *l*-carvone). As no new data on specifications were provided for (+)-carvone (No. 380.1) and (–)-carvone (No. 380.2), the existing specifications were maintained. Revisions were made to the chemical formulae for 3-[(2-methyl-3-furyl)thio]-2-butanone (No. 1525) and *O*-ethyl *S*-(2-furylmethyl)thiocarbonate (No. 1526), both of which remained as full specifications.

Full specifications were prepared for the 20 new flavouring agents for which data were provided. Forty-seven flavouring agents for which full specifications (Nos 427, 973–975, 980–982, 1480, 1491–1526 and 2103–2105) currently exist were considered by the Committee for toxicological re-evaluation at the current meeting (see sections 4.1.1, 4.1.3, 4.1.5 and 4.1.6). Thirty-nine of these flavouring agents (Nos. 1491–1526 and 2103–2105; see section 4.1.3) previously included a statement indicating that the safety evaluation for the flavouring agents had not been completed. As the toxicological evaluations of these 39 flavourings were completed at this meeting and no safety concerns were noted, the text indicating that the safety evaluation for these flavouring agents had not been completed was removed from the specifications and the specifications were maintained as full.

A statement was added to the existing full specifications for *p*-mentha-1,8-dien-7-al (perillaldehyde; No. 973), (+)-carvone (No. 380.1) and (–)-carvone (No. 380.2) indicating that, at the current meeting, the safety evaluations for these flavouring agents were not completed. The specifications for *p*-mentha-1,8-dien-7-al (No. 973), (+)-carvone (No. 380.1) and (–)-carvone (No. 380.2) were maintained as full.



#### 4.2.2 Revised specifications

The Committee received information in support of the revision of full specifications for three flavouring agents that were on the agenda of the present meeting (Nos 433, 619 and 2123).

The Committee revised specifications for *l*-menthyl lactate (No. 433) to reflect the specific isomeric composition of the flavouring agent in commerce. This was accomplished by changing the CAS number from 59259-38-0 to 61597-98-6 and revising the name to *l*-menthyl *l*-lactate.

The Committee revised specifications for *l*-malic acid (No. 619) by removing the specification for specific rotation based on results received, which indicate difficulty in standardizing this measurement for *l*-malic acid.

The Committee revised the specifications for glutamyl-valyl-glycine by revising the melting-point range of the flavouring.



## 5. Future work and recommendations

### Specific food additives (other than flavouring agents)

#### Anionic methacrylate copolymer

The Committee noted that there were insufficient data to reach a conclusion on the genotoxic potential of methacrylic acid, one of the residual monomers of AMC. Further studies to clarify the in vivo carcinogenic potential are required to complete the evaluation of AMC.

#### Citric and fatty acid esters of glycerol

The specifications of CITREM were made tentative, pending a suitable validated method for the determination of total citric acid content, along with performance characteristics of the method and data on the total citric acid content in at least five batches of products currently available in commerce, determined using that method.

The Committee noted that the method for total glycerol still uses chloroform. The Committee encouraged the submission of a method for total glycerol that eliminates the use of chloroform.

Specifications were revised and made tentative. Specifications will be withdrawn if suitable information is not provided by **December 2019**.

#### Neutral methacrylate copolymer

The Committee noted that no data were submitted for a suitable method of assay. Tentative specifications for NMC were prepared pending a suitable validated method of assay.

#### Spirulina extract

The Committee received limited analytical data on spirulina extract. In order to remove the tentative designation from the specifications, the following information on the products of commerce is requested by **December 2019**:

- Full compositional characterization of commercial products in both liquid and powder forms;
- Full compositional characterization of the aqueous extract before formulation/standardization;
- Validated analytical methods for identification of the substance with a suitable specificity (including validation data and representative batch data); and

- Validated analytical methods for the determination of the purity of the substance with a suitable specificity (including validation data and representative batch data).

## Modified starches

The Committee requested additional data and a suitable method for the determination of propylene chlorohydrins in Hydroxypropyl starch (INS 1440) and Hydroxypropyl distarch phosphate (INS 1442) in order to consider lowering this limit.

The Committee requested suitable microbiological acceptance criteria and supporting data for all modified starches. Information required in the recommended annexes is summarized in [Table 9](#).

## Flavouring agents

### Carvone and structurally related substances

For (+)-carvone (No. 380.1), the Committee concluded that a review of the ADI is recommended based on the evaluation of all biochemical and toxicological data. Also, data are needed for an exposure assessment for the oral exposure to (+)-carvone from all sources.

The ADI for (+)-carvone is maintained pending review of the ADI at a future meeting. The Committee recommends that the re-evaluation is completed within **3 years**.

For (–)-carvone (No. 380.2), the Committee concluded that toxicological data on (–)-carvone are necessary. Also, data are needed for an exposure assessment for the oral exposure to (–)-carvone from all sources.

### Maltol and related substances

The Committee could not verify the NOEL of 100 mg/kg bw in rats that was used to derive the ADI of 0–1 mg/kg bw for maltol (No. 1480) during its twenty-fifth meeting because of uncertainties in the administered dose levels and the effects observed in several studies described in the monograph of that meeting ([Annex 1](#), reference 57).

The Committee withdrew the ADI for maltol. The Committee concluded that access to either the original studies or submission of new data would be needed to reaffirm or amend the ADI.

The ADI for ethyl maltol was maintained.

## **Acknowledgements**

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



## Annex 1

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

1. General principles governing the use of food additives (First report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 15, 1957; WHO Technical Report Series, No. 129, 1957 (out of print).
2. Procedures for the testing of intentional food additives to establish their safety for use (Second report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 17, 1958; WHO Technical Report Series, No. 144, 1958 (out of print).
3. Specifications for identity and purity of food additives (antimicrobial preservatives and antioxidants) (Third report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as Specifications for identity and purity of food additives, Vol. I. Antimicrobial preservatives and antioxidants, Rome, Food and Agriculture Organization of the United Nations, 1962 (out of print).
4. Specifications for identity and purity of food additives (food colours) (Fourth report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as Specifications for identity and purity of food additives, Vol. II. Food colours, Rome, Food and Agriculture Organization of the United Nations, 1963 (out of print).
5. Evaluation of the carcinogenic hazards of food additives (Fifth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 29, 1961; WHO Technical Report Series, No. 220, 1961 (out of print).
6. Evaluation of the toxicity of a number of antimicrobials and antioxidants (Sixth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 31, 1962; WHO Technical Report Series, No. 228, 1962 (out of print).
7. Specifications for the identity and purity of food additives and their toxicological evaluation: emulsifiers, stabilizers, bleaching and maturing agents (Seventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 35, 1964; WHO Technical Report Series, No. 281, 1964 (out of print).
8. Specifications for the identity and purity of food additives and their toxicological evaluation: food colours and some antimicrobials and antioxidants (Eighth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 38, 1965; WHO Technical Report Series, No. 309, 1965 (out of print).
9. Specifications for identity and purity and toxicological evaluation of some antimicrobials and antioxidants. FAO Nutrition Meetings Report Series, No. 38A, 1965; WHO/Food Add/24.65 (out of print).
10. Specifications for identity and purity and toxicological evaluation of food colours. FAO Nutrition Meetings Report Series, No. 38B, 1966; WHO/Food Add/66.25.
11. Specifications for the identity and purity of food additives and their toxicological evaluation: some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases (Ninth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 40, 1966; WHO Technical Report Series, No. 339, 1966 (out of print).

12. Toxicological evaluation of some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases. FAO Nutrition Meetings Report Series, No. 40A, B, C; WHO/Food Add/67.29.
13. Specifications for the identity and purity of food additives and their toxicological evaluation: some emulsifiers and stabilizers and certain other substances (Tenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 43, 1967; WHO Technical Report Series, No. 373, 1967.
14. Specifications for the identity and purity of food additives and their toxicological evaluation: some flavouring substances and non-nutritive sweetening agents (Eleventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 44, 1968; WHO Technical Report Series, No. 383, 1968.
15. Toxicological evaluation of some flavouring substances and non-nutritive sweetening agents. FAO Nutrition Meetings Report Series, No. 44A, 1968; WHO/Food Add/68.33.
16. Specifications and criteria for identity and purity of some flavouring substances and non-nutritive sweetening agents. FAO Nutrition Meetings Report Series, No. 44B, 1969; WHO/Food Add/69.31.
17. Specifications for the identity and purity of food additives and their toxicological evaluation: some antibiotics (Twelfth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 45, 1969; WHO Technical Report Series, No. 430, 1969.
18. Specifications for the identity and purity of some antibiotics. FAO Nutrition Meetings Series, No. 45A, 1969; WHO/Food Add/69.34.
19. Specifications for the identity and purity of food additives and their toxicological evaluation: some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances (Thirteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 46, 1970; WHO Technical Report Series, No. 445, 1970.
20. Toxicological evaluation of some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances. FAO Nutrition Meetings Report Series, No. 46A, 1970; WHO/Food Add/70.36.
21. Specifications for the identity and purity of some food colours, emulsifiers, stabilizers, anticaking agents, and certain other food additives. FAO Nutrition Meetings Report Series, No. 46B, 1970; WHO/Food Add/70.37.
22. Evaluation of food additives: specifications for the identity and purity of food additives and their toxicological evaluation: some extraction solvents and certain other substances; and a review of the technological efficacy of some antimicrobial agents (Fourteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 48, 1971; WHO Technical Report Series, No. 462, 1971.
23. Toxicological evaluation of some extraction solvents and certain other substances. FAO Nutrition Meetings Report Series, No. 48A, 1971; WHO/Food Add/70.39.
24. Specifications for the identity and purity of some extraction solvents and certain other substances. FAO Nutrition Meetings Report Series, No. 48B, 1971; WHO/Food Add/70.40.
25. A review of the technological efficacy of some antimicrobial agents. FAO Nutrition Meetings Report Series, No. 48C, 1971; WHO/Food Add/70.41.
26. Evaluation of food additives: some enzymes, modified starches, and certain other substances: Toxicological evaluations and specifications and a review of the technological efficacy of some



- antioxidants (Fifteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 50, 1972; WHO Technical Report Series, No. 488, 1972.
27. Toxicological evaluation of some enzymes, modified starches, and certain other substances. FAO Nutrition Meetings Report Series, No. 50A, 1972; WHO Food Additives Series, No. 1, 1972.
  28. Specifications for the identity and purity of some enzymes and certain other substances. FAO Nutrition Meetings Report Series, No. 50B, 1972; WHO Food Additives Series, No. 2, 1972.
  29. A review of the technological efficacy of some antioxidants and synergists. FAO Nutrition Meetings Report Series, No. 50C, 1972; WHO Food Additives Series, No. 3, 1972.
  30. Evaluation of certain food additives and the contaminants mercury, lead, and cadmium (Sixteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 51, 1972; WHO Technical Report Series, No. 505, 1972, and corrigendum.
  31. Evaluation of mercury, lead, cadmium and the food additives amaranth, diethylpyrocarbamate, and octyl gallate. FAO Nutrition Meetings Report Series, No. 51A, 1972; WHO Food Additives Series, No. 4, 1972.
  32. Toxicological evaluation of certain food additives with a review of general principles and of specifications (Seventeenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 53, 1974; WHO Technical Report Series, No. 539, 1974, and corrigendum (out of print).
  33. Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers, and thickening agents. FAO Nutrition Meetings Report Series, No. 53A, 1974; WHO Food Additives Series, No. 5, 1974.
  34. Specifications for identity and purity of thickening agents, anticaking agents, antimicrobials, antioxidants and emulsifiers. FAO Food and Nutrition Paper, No. 4, 1978.
  35. Evaluation of certain food additives (Eighteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 54, 1974; WHO Technical Report Series, No. 557, 1974, and corrigendum.
  36. Toxicological evaluation of some food colours, enzymes, flavour enhancers, thickening agents, and certain other food additives. FAO Nutrition Meetings Report Series, No. 54A, 1975; WHO Food Additives Series, No. 6, 1975.
  37. Specifications for the identity and purity of some food colours, enhancers, thickening agents, and certain food additives. FAO Nutrition Meetings Report Series, No. 54B, 1975; WHO Food Additives Series, No. 7, 1975.
  38. Evaluation of certain food additives: some food colours, thickening agents, smoke condensates, and certain other substances (Nineteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 55, 1975; WHO Technical Report Series, No. 576, 1975.
  39. Toxicological evaluation of some food colours, thickening agents, and certain other substances. FAO Nutrition Meetings Report Series, No. 55A, 1975; WHO Food Additives Series, No. 8, 1975.
  40. Specifications for the identity and purity of certain food additives. FAO Nutrition Meetings Report Series, No. 55B, 1976; WHO Food Additives Series, No. 9, 1976.

41. Evaluation of certain food additives (Twentieth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Food and Nutrition Meetings Series, No. 1, 1976; WHO Technical Report Series, No. 599, 1976.
42. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 10, 1976.
43. Specifications for the identity and purity of some food additives. FAO Food and Nutrition Series, No. 1B, 1977; WHO Food Additives Series, No. 11, 1977.
44. Evaluation of certain food additives (Twenty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 617, 1978.
45. Summary of toxicological data of certain food additives. WHO Food Additives Series, No. 12, 1977.
46. Specifications for identity and purity of some food additives, including 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/pes](http://www.who.int/pes).
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, 2018 (in press).
236. Compendium of food additive specifications. FAO JECFA Monographs 20, 2017.
237. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 75, 2018 (in press).

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



## ANNEX 2

### Toxicological information and information on specifications

#### *Food additives evaluated toxicologically and assessed for dietary exposure*

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions
Anionic methacrylate copolymer (AMC)	N, T <sup>a</sup>	<b>The Committee was unable to complete the evaluation of AMC.</b> While the copolymer itself is not of health concern, genotoxicity concerns remains for the residual monomer methacrylic acid. The specifications were made tentative pending the completion of the safety evaluation of AMC.
Basic methacrylate copolymer (BMC)	N	<b>The Committee established an ADI “not specified” for basic methacrylate copolymer.</b>  The Committee concluded that the use of BMC that complies with the specifications established at the current meeting is not of safety concern when the food additive is used as a coating or glazing agent for solid food supplements and for foods for special medical purposes and micronutrient encapsulation for food fortification. The no-observed-adverse-effect level (NOAEL) for BMC ranged from 750 to 2 000 mg/kg body weight (bw) per day, which were the highest doses tested.  The Committee evaluated exposure to BMC for the copolymer and its monomers ( <i>n</i> -butyl methacrylate, 2-(dimethylamino)ethyl methacrylate and methyl methacrylate). Estimated exposures to BMC range from 3.0 to 135 mg/kg bw per day. The total monomeric content of BMC is less than 0.3%. The Committee concluded that the toxicological data on the residual monomers do not give rise to concerns when taking into account the low dietary exposures.
Erythrosine	R <sup>b</sup>	<b>The Committee concluded that the new data that have become available since the previous evaluation of erythrosine do not give reason to revise the ADI and confirmed the previous ADI of 0–0.1 mg/kg bw.</b>  The Committee noted that the dietary exposure estimate for erythrosine of 0.09 mg/kg bw per day (95th percentile for children) was close to the upper bound of the ADI. Given that this estimate of exposure is for children and it is a high percentile for consumers only, such a level is unlikely to occur every day over a lifetime. Therefore, the Committee concluded that dietary exposures to for all age groups do not present a health concern. erythrosine for all age groups do not present a health concern.
Indigotine	R <sup>b</sup>	The Committee considered the new data that had become available since the previous evaluation as well as previously evaluated studies and <b>concluded that there are no reasons to revise the ADI and confirmed the previous ADI of 0–5 mg/kg bw.</b>

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions
Lutein	R <sup>c,d</sup>	<p>The Committee noted that the conservative dietary exposure estimate of 0.8 mg/kg bw per day (95th percentile for children and toddlers) is less than the upper bound of the ADI of 0–5 mg/kg bw. The Committee concluded that dietary exposure to indigotine for all age groups does not present a health concern.</p> <p>Free lutein, lutein esters and free zeaxanthin including <i>meso</i>-zeaxanthin are biochemically and toxicologically equivalent. At the present meeting the Committee concluded that there were sufficient toxicological data to complete a safety assessment of lutein and lutein esters from <i>Tagetes erecta</i>, synthetic zeaxanthin and <i>meso</i>-zeaxanthin. Free lutein, lutein esters and free zeaxanthin and <i>meso</i>-zeaxanthin are substances of low toxicity for which no adverse effects have been observed in a broad range of toxicological studies in laboratory animals and clinical studies in humans.</p> <p>Based on the absence of toxicity in a wide range of studies, <b>the Committee established a group ADI “not specified” for lutein from <i>Tagetes erecta</i>, lutein esters from <i>Tagetes erecta</i> and zeaxanthin (synthetic).</b></p> <p><i>Meso</i>-zeaxanthin was not included in this group ADI, as specifications are not currently available.</p> <p><b>The group ADI of 0–2 mg/kg bw for lutein from <i>Tagetes erecta</i> and zeaxanthin (synthetic) was withdrawn.</b></p>
Neutral methacrylate copolymer (NMC)	N, T	<p><b>The Committee established an ADI “not specified” for NMC. The ADI “not specified” was made temporary because the specifications are tentative.</b></p> <p>The Committee concluded that the use of NMC that complies with the specifications established at the current meeting is not of safety concern when the food additive is used as a coating or glazing agent for solid food supplements and for foods for special medical purposes. The NOAELs for NMC ranged from 454 to 2 000 mg/kg bw per day, and these were the highest doses tested.</p> <p>The Committee evaluated exposure to NMC for the copolymer and its monomers (methyl methacrylate and ethyl acrylate). Estimated exposures to NMC range from 5.8 to 86 mg/kg bw per day. The total monomeric content of NMC is less than 0.01%. Toxicological data on the residual monomers do not give rise to concerns when taking into account the low dietary exposures.</p>
Sorbitol syrup	–	<p>Sorbitol syrup (INS 420(ii)) is currently included in the Codex General Standard for Food Additives (GSFA) although it has not been assigned an ADI or determined, on the basis of other criteria, to be safe. The Committee was therefore requested to consider the previous evaluations of sorbitol, hydrogenated glucose syrups and other relevant substances, and advise on the need for a separate evaluation of sorbitol syrup or if the ADI “not specified” for sorbitol is also applicable for sorbitol syrup.</p>

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions
		Based on the similarity of the chemical constituents of sorbitol syrup to the previously evaluated sorbitol, maltitol syrup and polyglycolic syrup, <b>the Committee concluded that there is no need for a separate evaluation of sorbitol syrup and established an ADI “not specified” for sorbitol syrup.</b>
Spirulina extract	N, T	<p><b>The Committee established a temporary ADI “not specified” for spirulina extract.</b> The ADI was based on the absence of toxicity in repeated-dose animal studies with spirulina extract and dried spirulina. The ADI “not specified” was made temporary due to the tentative nature of the specifications.</p> <p>Expressed as phycocyanins, estimated dietary exposure from the use of spirulina extract as a food colour based on the Budget method and exposure to spirulina extract and dried spirulina from other dietary sources, including food ingredients, dietary supplements, and coatings of food supplements was 190 mg/kg bw for adults (60 kg/person) and 650 mg/kg bw for a child (15 kg/person). The Committee concluded that this dietary exposure does not present a health concern.</p>

—: no specifications prepared; N: new specifications; R: existing specifications revised; T: tentative specifications

<sup>a</sup> The specifications were made tentative pending the completion of the safety evaluation of AMC.

<sup>b</sup> At the current meeting, high-performance liquid chromatographic (HPLC) methods were added for determining subsidiary colouring matters and organic compounds other than colouring matters. The method of assay was changed to visible spectrophotometry, and spectrophotometric data were provided for the colour dissolved in water.

<sup>c</sup> The specifications for lutein esters from *Tagetes erecta* and zeaxanthin (synthetic) were maintained.

<sup>d</sup> At the current meeting, the identity test for melting range was deleted, the identity tests for carotenoids and spectrophotometry were updated, the test for propylene glycol was incorporated verbatim and the previous reference removed, and the method of assay was updated.

### Food additives considered for specifications only

Food additive	Specifications
Cassia gum	R <sup>a</sup>
Citric and fatty acid esters of glycerol	R, T <sup>b</sup>
Glycerol ester of wood rosin	R <sup>c</sup>
Modified starches	R <sup>d</sup> , T

R: existing specifications revised; T: tentative specifications

<sup>a</sup> The Committee, at its current meeting, received analytical methods and included the most suitable validated method in the specifications monograph. However, this method uses chloroform for the extraction of anthraquinones. Extraction with *n*-hexane and diethyl ether resulted in poor recovery of anthraquinones. The Committee recommends that the JECFA Secretariat be notified if an alternative extraction solvent is identified. The specifications were revised and the tentative status was removed.

<sup>b</sup> The Committee did not receive a replacement method for the obsolete packed column gas chromatographic method for the determination of total citric acid, in its specifications monograph. The Committee noted further that the method for total glycerol still uses chloroform. The Committee encouraged the submission of a method for total glycerol that eliminates the use of chloroform. Specifications were revised and made tentative pending the availability of data. Specifications will be withdrawn if suitable information is not provided by December 2019.

<sup>c</sup> The Committee received information on the manufacture of GEWR from the rosin obtained from the stumps of two additional species namely *Pinus halepensis* and *Pinus brutia* as source materials. Recognizing the natural variability of the composition of wood rosin, the Committee removed the restriction to certain pine species within the specifications. Since the specifications monograph for GEWR does not contain an assay, the Committee recommends that the JECFA Secretariat be notified upon the development and validation of an appropriate assay. The existing specifications were revised.

<sup>d</sup> The Committee reviewed data on the method of manufacture, identity, and purity of all 16 modified starches. Based on the information received, and available information the Committee noted the following:

- All processes are performed under similar manufacturing conditions and result in minor chemical modifications. Given the chemical and physical similarities of modified starches, the Committee at previous meetings considered the application of a read-across approach to be appropriate for the toxicological evaluation of these substances.
- All 16 modified starches had been assigned an ADI of “not specified”.

- All modified starches can be additionally bleached or fragmented; therefore revision in the specifications of bleached or fragmented starches would imply the revision of all 16 monographs.
- Microbiological specifications were not present in the existing specifications for all modified starches.
- Several specifications were common to all modified starches (such as for heavy metals impurities content and microbiological considerations). Revision of those common specifications would affect all 16 monographs.
- As a result of the wide range of products manufactured, the identification tests required to unambiguously chemically characterize each modified starch in individual specifications may be cumbersome, potentially unavailable, and unlikely to reflect market requirements.
- It may not be possible to publish identification tests based on market requirements without unduly revealing proprietary information.
- Based on the points noted above, individual specifications for several modified starches may remain tentative for an indefinite period or may need to be withdrawn.

The Committee therefore recommended that a new approach to the specifications monographs should be introduced to account for the chemical similarity between all modified starches, their functional diversity, the variety of chemicals used in their manufacture, and the corresponding diversity of impurities. The Committee recommended that all modified starches be included in a modular monograph titled 'Modified Starches' that contains common requirements [General specifications for modified starches] consisting of specifications that apply to all 16 modified starches (INS 1400, 1401, 1402, 1403, 1404, 1405, 1410, 1412, 1413, 1414, 1420, 1422, 1440, 1442, 1450, 1451), and annexes with specifications applicable to each individual modified starch based on the treatment(s) received. The Committee drafted a new modular specifications monograph titled "Modified starches" consisting of an explanatory introduction, "General specifications for modified starches," and eight annexes. The new modular specifications monograph for modified starches is printed in FAO Monograph 22, and will replace the 16 existing individual specifications for modified starches (INS 1400, 1401, 1402, 1403, 1404, 1405, 1410, 1412, 1413, 1414, 1420, 1422, 1440, 1442, 1450, 1451).

The specification for lead included in the General specifications be decreased from 2 mg/kg to 0.2 mg/kg. The limit of lead for starch sodium octenylsuccinate for use in infant formula and formula for special medical purposes intended for infants was set to 0.1 mg/kg in the General specifications.

The methods for the determination of free adipic acid and adipate groups, residual vinyl acetate, free octenyl succinic acid and octenyl succinate esters were revised and a method for the determination of propylene chlorohydrins was added.

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

### A. Alicyclic primary alcohols, aldehydes, acids and related esters

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
<b>Structural class I</b>			
Mixture of 1-Vinyl-3- cyclohexenecarbaldehyde and 4-vinyl-1-cyclohexenecarbaldehyde	2253	N	No safety concern
<i>p</i> -Mentha-1,8-dien-7-ol	974	N	No safety concern
<i>p</i> -Mentha-1,8-dien-7-yl acetate	975	N	No safety concern
Formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene	980	N	No safety concern
Myrtenol	981	N	No safety concern
Myrtenyl acetate	982	M	No safety concern
<b>Structural class II</b>			
(1-Methyl-2-(1,2,2- trimethylbicyclo[3.1.0]hex-3-ylmethyl)cyclopropyl)methanol	2254	N	No safety concern
<b>Structural class III</b>			
(±)-Bicyclo[2.2.1]hept-5-ene-2-carboxylic acid, ethyl ester	2255	N	No safety concern
<b>Flavouring agent excluded at Step 1 of the Procedure</b>			
<i>p</i> -Mentha-1,8-dien-7-al (Perillaldehyde)	973	M	Genotoxicity data for <i>p</i> -mentha-1,8-dien-7-al raise concerns for potential genotoxicity

M: existing specifications maintained; N: new specifications



**B. Carvone and structurally related substances**

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
<b>Structural class I</b>			
Pinocaryyl isobutyrate	2242	N	No safety concern
Carvyl palmitate	2243	N	No safety concern
<b>Structural class III</b>			
6-Hydroxycarvone	2244	N	No safety concern
<b>Flavouring agents not evaluated according to the revised Procedure</b>			
(+)-Carvone	380.1	M	The Committee did not re-evaluate (+)-carvone (No. 380.1) according to the revised Procedure given the lack of information on the oral exposure from all sources and the need to review the ADI. A review of the ADI is recommended based on the evaluation of all biochemical and toxicological data. Also, data are needed for an exposure assessment for oral exposure to (+)-carvone from all sources to complete the evaluation for (+)-carvone.
(-)-Carvone	380.2	M	The Committee did not re-evaluate (-)-carvone (No. 380.2) according to the revised Procedure given the lack of information on the oral exposure from all sources and the lack of toxicological data.

M: existing specifications maintained; N: new specifications

**C. Furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers**

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
<b>Structural class III</b>			
2-Pentylfuran	1491	M <sup>a</sup>	No safety concern
2-Heptylfuran	1492	M <sup>a</sup>	No safety concern
2-Decylfuran	1493	M <sup>a</sup>	No safety concern
3-Methyl-2-(3-methylbut-2-enyl)-furan	1494	M <sup>a</sup>	No safety concern
2,3-Dimethylbenzofuran	1495	M <sup>a</sup>	No safety concern
2,4-Difurfurylfuran	1496	M <sup>a</sup>	No safety concern
3-(2-Furyl)acrolein	1497	M <sup>a</sup>	No safety concern
2-Methyl-3(2-furyl)acrolein	1498	M <sup>a</sup>	No safety concern
3-(5-Methyl-2-furyl)prop-2-enal	1499	M <sup>a</sup>	No safety concern
3-(5-Methyl-2-furyl)butanal	1500	M <sup>a</sup>	No safety concern
2-Furfurylidene-butyraldehyde	1501	M <sup>a</sup>	No safety concern

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
2-Phenyl-3-(2-furyl)prop-2-enal	1502	M <sup>a</sup>	No safety concern
2-Furyl methyl ketone	1503	M <sup>a</sup>	No safety concern
2-Acetyl-5-methylfuran	1504	M <sup>a</sup>	No safety concern
2-Acetyl-3,5-dimethylfuran	1505	M <sup>a</sup>	No safety concern
3-Acetyl-2,5-dimethylfuran	1506	M <sup>a</sup>	No safety concern
2-Butyrylfuran	1507	M <sup>a</sup>	No safety concern
(2-Furyl)-2-propanone	1508	M <sup>a</sup>	No safety concern
2-Pentanoylfuran	1509	M <sup>a</sup>	No safety concern
1-(2-Furyl)butan-3-one	1510	M <sup>a</sup>	No safety concern
4-(2-Furyl)-3-buten-2-one	1511	M <sup>a</sup>	No safety concern
Pentyl 2-furyl ketone	1512	M <sup>a</sup>	No safety concern
Ethyl 3-(2-furyl)propanoate	1513	M <sup>a</sup>	No safety concern
Isobutyl 3-(2-furan)propionate	1514	M <sup>a</sup>	No safety concern
Isoamyl 3-(2-furan)propionate	1515	M <sup>a</sup>	No safety concern
Isoamyl 3-(2-furan)butyrate	1516	M <sup>a</sup>	No safety concern
Phenethyl 2-furoate	1517	M <sup>a</sup>	No safety concern
Propyl 2-furanacrylate	1518	M <sup>a</sup>	No safety concern
2,5-Dimethyl-3-oxo-(2H)-fur-4-yl butyrate	1519	M <sup>a</sup>	No safety concern
Furfuryl methyl ether	1520	M <sup>a</sup>	No safety concern
Ethyl furfuryl ether	1521	M <sup>a</sup>	No safety concern
Difurfuryl ether	1522	M <sup>a</sup>	No safety concern
2,5-Dimethyl-3-furanthiol acetate	1523	M <sup>a</sup>	No safety concern
Furfuryl 2-methyl-3-furyl disulfide	1524	M <sup>a</sup>	No safety concern
3-[(2-Methyl-3-furyl)thio]-2-butanone	1525	M <sup>a</sup>	No safety concern
O-Ethyl-S-(2-furylmethyl)thiocarbonate	1526	M <sup>a</sup>	No safety concern
(E)-Ethyl 3-(2-furyl)acrylate	2103	M <sup>a</sup>	No safety concern
di-2-Furylmethane	2104	M <sup>a</sup>	No safety concern
2-Methylbenzofuran	2105	M <sup>a</sup>	No safety concern

M: existing specifications maintained

<sup>a</sup> The text indicating that the safety evaluation for these flavouring agents had not been completed was removed from the specifications and the specifications were maintained as full.

#### **D. Linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters**

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
<b>Structural class I</b>			
<i>trans</i> -6-Octenal	2240	N	No safety concern
2,6-Dimethyl-5-heptenol	2241	N	No safety concern

N: new specifications

**E. Maltol and related substances**

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
<b>Structural class II</b>			
Maltol	1480	M	No safety concern <sup>a</sup>
<b>Structural class III</b>			
Ethyl maltol isobutyrate	2252	N	No safety concern

M: existing specifications maintained; N: new specifications

<sup>a</sup> The previously established ADI for maltol was withdrawn by the Committee.

**F. Menthol and structurally related substances**

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
<b>Structural class I</b>			
Menthyl formate	2246	N	No safety concern
Menthyl propionate	2247	N	No safety concern
<i>l</i> -Menthyl butyrate	2248	N	No safety concern
<i>dl</i> -Isomenthol	2249	N	No safety concern
Dimethyl glutarate	2250	N	No safety concern
Menthol	427	M	No safety concern
<b>Structural class III</b>			
(±)-2-[(2- <i>p</i> -Methoxy)ethoxy]ethanol	2251	N	No safety concern

M: existing specifications maintained; N: new specifications

<sup>a</sup> The ADI of menthol of 0–4 mg/kg bw established at the fifty-first meeting was maintained.

**G. Miscellaneous nitrogen-containing substances**

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
<b>Structural class III</b>			
2-(((3-(2,3-Dimethoxyphenyl)-1 <i>H</i> -1,2,4-triazol-5-yl)thio)methyl)pyridine	2235	N	No safety concern
( <i>S</i> )-1-(3-(((4-Amino-2,2-dioxido-1 <i>H</i> -benzo[ <i>c</i> ][1,2,6]thiadiazin-5-yl)oxy)methyl)piperidin-1-yl)-3-methylbutan-1-one	2236	N	No safety concern
2-(4-Methylphenoxy)- <i>N</i> -(1 <i>H</i> -pyrazol-3-yl)- <i>N</i> -(thiophen-2-ylmethyl)acetamide	2237	N	No safety concern

N: new specifications

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

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
<b>Structural class I</b>			
8-Methyldecanal	2238	N	No safety concern
8-Methylnonanal	2239	N	No safety concern

N: new specifications

**Food additives considered for specifications only**

Flavouring agent	No.	Specifications
L -Menthyl lactate	433	R <sup>a</sup>
L -Malic acid	619	R <sup>b</sup>
Glutamyl-valyl-glycine	2123	R <sup>c</sup>

R: existing specifications revised

<sup>a</sup> The CAS number was changed from 59259-38-0 to 61597-98-6 and the name to *l*-menthyl *l*-lactate.

<sup>b</sup> The specification for specific rotation were removed.

<sup>c</sup> The melting point range was revised.

## Annex 3

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

JECFA No.	Flavouring agent	Minimum assay value	Secondary components	Comments on secondary components
<b>Furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers</b>				
1519	2,5-Dimethyl-3-oxo-(2 <i>H</i> )-furyl butyrate	93%	4-Hydroxy-2,5-dimethyl-3(2 <i>H</i> )-furanone (1–3%)  Butyric acid (No. 87) (1–3%)	The SPET value for No. 1519 is 1 200 µg/day, and 3% of this value is 36 µg/day, which is below the class III threshold of toxicological concern.  Butyric acid (No. 87) has previously been evaluated by the Committee to be of no safety concern at estimated dietary exposures when used as a flavouring agent.
1524	Furfuryl 2-methyl-3-furyl disulfide	90%	Di-(2-methyl-3-furyl) disulfide (6–7%)	The SPET value for No. 1524 is 10 µg/day, and 7% of this value is 0.7 µg/day, which is below the class III threshold of toxicological concern. The major secondary component of No. 1524 is therefore not considered to present a safety concern at estimated dietary exposures from the use of No. 1524 as a flavouring agent.
<b>Linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters</b>				
2241	2,6-Dimethyl-5-heptenol	>90%	2,6-Dimethyl-5-heptenal (No. 349) (1–6%)	2,6-Dimethyl-5-heptenal (No. 349) has previously been evaluated by the Committee to be of no safety concern at estimated dietary exposures when used as a flavouring agent.
<b>Maltol and related substances</b>				
2252	Ethyl maltol isobutyrate	93%	Ethyl maltol (No. 1481) (2–3%)	Ethyl maltol (No. 1481) has previously been evaluated by the Committee to be of no safety concern at estimated dietary exposures when used as a flavouring agent.



# Annex 4

## Meeting agenda



Food and Agriculture  
Organization of the  
United Nations



World Health  
Organization

86<sup>th</sup> JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES (JECFA)  
WHO Headquarters, Geneva 12 – 21 June 2018

### Draft Agenda

1. Opening
2. Declarations of Interests (information by the Secretariat on any declared interests and discussion, update by experts)
3. Election of Chairperson and Vice-Chairperson, appointment of Rapporteurs
4. Adoption of Agenda
5. Matters of interest arising from previous Sessions of the Codex Committee on Food Additives (CCFA)
6. Critical issues and questions from Working Papers (first brief round of discussion on all subjects to inform the full committee)
7. Evaluations

#### Food Additives

7.1. Toxicological Evaluation, Exposure Assessment, and Establishment of Specifications:

- Basic methacrylate copolymer (INS 1205)
- Neutral methacrylate copolymer (INS 1206)
- Anionic methacrylate copolymer (INS 1207)
- Lutein from *Tagetes erecta* (INS 161b(i))
- Spirulina extract
- Erythrosine (INS 127)
- Indigotine (INS 132)

7.2. Food additives for revision of specifications and analytical methods:

- Citric and fatty acid esters of glycerol (INS 472 c)
- Glycerol ester of wood rosin (GEWR) (INS445(iii))
- Cassia gum
- Dextrin roasted starch (INS 1400)
- Acid treated starch (INS 1401)
- Alkaline treated starch (INS 1402)
- Bleached starch (INS1403)
- Enzyme-treated starch (INS 1405)
- Monostarch phosphate (INS 1410)
- Distarch phosphate (INS 1412)
- Phosphated distarch phosphate (INS 1413)
- Acetylated distarch phosphate (INS 1414)
- Acetylated distarch adipate (INS 1422)
- Hydroxypropyl starch (INS 1440)
- Hydroxypropyl distarch phosphate (INS 1442)
- Starch sodium octenyl succinate (INS 1450)

Flavourings

7.3 Toxicological evaluation, exposure assessment and establishment of specifications for certain flavourings

- Miscellaneous nitrogen-containing substances
- Saturated aliphatic acyclic branched-chain primary alcohols, aldehydes, and acids
- Linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids, and related esters
- Carvone and structurally related substances
- Menthol and structurally related substances
- Maltol and related substances
- Alicyclic primary alcohols, aldehydes, acids and related esters (reevaluation)
- Furan substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers.(reevaluation)

8. Revision of specification for certain flavourings

9. Other matters to be considered (general considerations)

Update of EHC240:

- Development on guidance on the evaluation of genotoxicity studies
- Updated guidance on dose-response modelling for the use in risk assessment
- Steviol glycosides and frame specifications/frame toxicological profiles

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

11. Adoption of the report.



## SELECTED WHO PUBLICATIONS OF RELATED INTEREST

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### **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**

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**

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)

### **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)

### **Toxicological Evaluation of Certain Veterinary Drug Residues in Food**

Eighty-first Meeting of the Joint FAO/WHO Expert Committee on Food Additives  
WHO Food Additives Series, No. 72, 2016 (162 pages)

### **Evaluation of Certain Food Additives and Contaminants**

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

### **Safety Evaluation of Certain Food Additives and Contaminants**

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

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## Evaluation of certain food additives

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

The first part of the report includes updates on the work of the Codex Committee on Food Additives (CCFA) since the eighty-fourth meeting of JECFA and on activities relevant to JECFA with regard to the *Environmental Health Criteria 240: Principles and methods for the risk assessment of chemicals in food* (EHC 240). Following is a summary of the Committee's evaluations of technical, toxicological and dietary exposure data for eight food additives other than flavouring agents – anionic methacrylate copolymer; basic methacrylate copolymer; erythrosine; indigotine; lutein and lutein esters from *Tagetes erecta* and zeaxanthin (synthetic); neutral methacrylate copolymer; sorbitol syrup; and spirulina extract – and eight groups of flavouring agents – alicyclic primary alcohols, aldehydes, acids and related esters; carvone and structurally related substances; furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers; linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters; maltol and related substances; menthol and structurally related substances; miscellaneous nitrogen-containing substances; and saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids.

Specifications and analytical methods were revised for the following food additives other than flavouring agents: cassia gum; citric and fatty acid esters of glycerol (CITREM); glycerol ester of wood rosin (GEWR); and modified starches.

Annexed to the report are tables summarizing the Committee's recommendations for dietary exposures to all of the food additives as well as toxicological information, dietary exposures and information on specifications.

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