



Toxicological profile for Oxidized starch

This ingredient has been assessed to determine potential human health effects for the consumer. It was considered not to increase the inherent toxicity of the product and thus is acceptable under conditions of intended use.

1. Name of substance and physico-chemical properties

1.1. IUPAC systematic name

2-[2-[[3,4-Dihydroxy-6-(hydroxymethyl)-5-methoxyoxan-2-yl]oxymethyl]-6-[4,5-dihydroxy-2-(hydroxymethyl)-6-methyloxan-3-yl]oxy-4,5-dihydroxyoxan-3-yl]oxy-4-hydroxy-6-(hydroxymethyl)-5-methoxyoxan-3-one (PubChem)

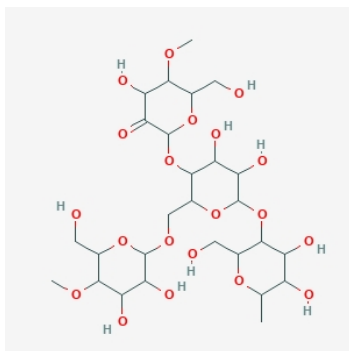
1.2. Synonyms

Starch, oxidized; Oxidized Natural Starch; Oxidized corn starch; Starch, bleached; oxidized starch; (PubChem)

1.3. Molecular formula

C₂₇H₄₆O₂₀ (PubChem)

1.4. Structural Formula



(PubChem)

1.5. Molecular weight (g/mol)

~10⁶ – 10⁸; 10⁴-10⁶ (ECCC, 2018)

1.6. CAS registration number

65996-62-5

1.7. Properties

1.7.1. Melting point

(°C): No data available to us at this time.

1.7.2. Boiling point

(°C): No data available to us at this time.

1.7.3. Solubility

Almost insoluble; Negligible to low (ECCC, 2018)

1.7.4. pKa

No data available to us at this time.

1.7.5. Flashpoint

(°C): No data available to us at this time.

1.7.6. Flammability limits (vol/vol%)

No data available to us at this time.

1.7.7. (Auto)ignition temperature

(°C): No data available to us at this time.

1.7.8. Decomposition temperature

(°C): No data available to us at this time.

1.7.9. Stability

Stable at normal temperatures and pressure.

1.7.10. Vapor pressure

"Negligible" (ECCC, 2018)

1.7.11. log Kow

-7.1 (estimated) (PubChem)

2. General information

2.1. Exposure

"The primary use for oxidized starch in Canada, according to information reported in the above mentioned surveys is as a plating agent, adhesive, sealant, and in paper and textile industries. It is also used in the toy industry as a viscosity reducer. In Canada, modified starch (including oxidized starch) can be used as a food ingredient in food products as long as the starch has been prepared from permitted starch-modifying agents as listed in the List of Permitted Starch-Modifying Agents. It has also been identified as a component in the manufacture of certain food packaging materials (personal communication, emails from Food Directorate, Health Products and Food Branch, Health Canada, dated December, 2016; unreferenced)."

"Globally, the primary use of oxidized starch is as a food additive (emulsifier, binder, thickening agent, and stabilizer) (FAO-WHO). Oxidized starch is also used globally in paper and textile industries"

As taken from ECCC, 2018

"The combined exposure to E 1404–E 1451 at the 95th percentile of the refined (brand-loyal) exposure assessment scenario for the general population was up to 3,053 mg/kg bw per day."

As taken from EFSA, 2017

Oxidized starch (CAS RN 65996-62-5) is used as a binder, emulsifying agent, stabilizing agent and thickening agent in non-medicinal natural health products (Health Canada, 2021).

2.2. Combustion products

No data available to us at this time.

2.3. Ingredient(s) from which it originates

“Whatever oxidizing agent is used only minor modifications of the starch molecule are achieved in normal manufacturing practice. These are equivalent to the introduction of 1% w/w of carboxyl (-COOH) or 0.5% w/w of keto (-CO) groups or, 3.6 carboxyl and 2.9 carbonyl groups per 100 glucopyranose units. No chlorine is introduced into the molecule and the final products usually contain only residues of sodium chloride, sodium sulfate and sulfur dioxide.”

As taken from WHO FOOD ADDITIVES SERIES NO. 5 available at <http://www.inchem.org/documents/jecfa/jecmono/v05je74.htm>

“Although oxidized starch does not occur naturally in the environment, the native starch is widely distributed in nature, specifically in plants.”

As taken from ECCC, 2018

3. Status in legislation and other official guidance

Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives OXIDIZED STARCH	
See:	MODIFIED STARCHES
INS:	1404
Chemical names:	OXIDIZED STARCH
Synonyms:	STARCH, OXIDIZED
Functional class:	EMULSIFIER; THICKENING AGENT; BINDER
Latest evaluation:	1982
ADI:	NOT SPECIFIED
Report:	TRS 683-JECFA 26/29
Specifications:	FNP 49-JECFA 35/28 (1989); COMPENDIUM/965
Tox monograph:	FAS 6/NMRS 54A-JECFA 18/146 (1974)
Previous status:	1988, FNP 38-JECFA 33/142. R,T 1985, FNP 34-JECFA 29/137. R 1983, FNP 28-JECFA 27/76. R 1982, FNP 25-JECFA 26/121. R

	1974, NMRS 54/TRS 557-JECFA 18/20, FAS 6/NMRS 54A-JECFA 18/146. ADI NOT SPECIFIED. NS. S 1973, NMRS 53/TRS 539-JECFA 17/22, FNP 4-JECFA 17/60, FAS 5/NMRS 53A-JECFA 17/365. ADI NOT LIMITED (TEMPORARY). NL/TE. R,T 1971, NMRS 50/TRS 488-JECFA 15/15, FAS 2/NMRS 50B-JECFA 15/45, FAS 1/NMRS 50A-JECFA 15/23. ADI NOT LIMITED (TEMPORARY). NL/TE. R 1969, NMRS 46/TRS 445-JECFA 13/13, FAS 70.37/NMRS 46B-JECFA 13/19, FAS 70.36/NMRS 46A-JECFA 13/66. ADI NOT LIMITED (TEMPORARY). NL/TE. N

As taken from JECFA, 2001, evaluation of Oxidized Starches available at http://www.inchem.org/documents/jecfa/jecval/jec_1803.htm

General Information

Synonyms:	STARCH, OXIDIZED
Chemical Names:	OXIDIZED STARCH
INS:	1404
Functional Class:	Food Additives EMULSIFIER THICKENER

Evaluations

Evaluation year:	2018
Comments:	Considered for specification only.
Meeting:	86
Specs Code:	R, T
Report:	TRS 1014-JECFA 86/65
Specification:	FAO JECFA Monographs 22/53
Evaluation year:	2016
ADI:	NOT SPECIFIED
Meeting:	82
Specs Code:	R
Report:	TRS 1000-JECFA 82/83
Specification:	FAO JECFA Monographs 19/132

As taken from WHO, 2021.

“Starch, food, modified: oxidized starches” is included on the FDA’s inventory of Substances Added to Food (formerly EAFUS) as a stabilizer or thickener, and is included under 21 CFR sections 172.892 (Food starch-modified) and 175.105 (adhesives) (FDA, 2022, 2023).

Starch, oxidised (CAS RN 65996-62-5) is not registered under REACH (ECHA, undated).

Starch, oxidised (CAS RN 65996-62-5) is not classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2023).

Starch, oxidized (CAS RN 65996-62-5) is listed in the US EPA Toxic Substances Control Act (TSCA) inventory and also on the US EPA 2012 CDR, 2020 CDR TSCA Inv Active and 2020 CDR Full Exempt lists (Chemical Data Reporting Rule). The CDR regulation requires companies that manufacture (including import) certain chemicals at certain volumes in the U.S. to report to EPA every four years through its CDR.

The TSCA inventory, 2020 CDR TSCA Inv Active, and 2012 CDR and 2020 CDR Full Exempt lists.

Oxidised starch (E1404) is authorized for use as a food additive in the EU under (EU) legislation no 1129/2011 and, as a Group I Additive, also under (EU) legislation nos 438/2013, 2015/0647, 2015/1832 and 2018/1497 (European Commission, 2011, 2019).

“Following the conceptual framework for the risk assessment of certain food additives, the Panel concluded that there is no safety concern for the use of modified starches as food additives at the reported uses and use levels for the general population and that there is no need for a numerical ADI.”

“The aforementioned modified starches [including oxidised starch] are authorised as food additives in accordance with Regulation (EC) No 1333/2008.”.

As taken from EFSA, 2017

Starch, oxidized (CAS RN 65996-62-5) “poses no unreasonable risk to human health based on Tier I assessment under the NICNAS IMAP assessment framework” (AICIS, 2019).

4. Metabolism/Pharmacokinetics

4.1. Metabolism/metabolites

“Early comparative experiments pointed to inhibition of amylolysis (Tremolières et al., 1959). In vitro digestibility by pancreatin or saliva was used to compare lightly and highly oxidized corn starch with unmodified corn starch and a reference starch. Maltose production after a fixed interval of enzyme action was taken as a measure of digestibility. The oxidized starch was 10-15 per cent. less digestible by pancreatic than unmodified starch but there was no obvious difference as regards salivary digestion (Shuman, A. C. & Mertz, E. T. 1959). The digestibility of oxidized wheat starch (conditions not stated) was examined in rats by matched-feeding techniques using the modified starch as the sole source of carbohydrate at a level of 63.7 per cent. (dry basis) of the diet. The degree of assimilation by and the general effects on groups of 6 rats over a feeding period of 28 days were assessed from consideration of body weight changes, faecal residues, digestibility coefficients for starch and post-mortem appearance of the animals and their gastrointestinal tracts. The digestibility coefficients were calculated from the starch content of ingested food and residues found in faeces and post-mortem gastrointestinal contents. Body weight gain and digestibility coefficients were practically indistinguishable from those obtained for wheat starch or corn starch. Nothing abnormal was noted on post-mortem examination (Booher et al., 1951).”

As taken from FAO Nutrition Meetings Report Series 46a, available at <http://www.inchem.org/documents/jecfa/jecmono/v46aje26.htm>

“In humans, modified starches are not absorbed intact but significantly hydrolysed by intestinal enzymes and then fermented by the intestinal microbiota.”

“Data on in vitro degradation of modified starches by digestive enzymes indicated that their digestibility was slightly reduced or showed no differences when compared to corresponding unmodified starches. In vivo data are in agreement with in vitro studies indicating that the two major components of starches, amylose and amylopectin, would be fermented during their passage through the large intestine by strains of bacteria found in the human colon. The main end products of this colonic anaerobic digestive process are short-chain fatty acids (SCFA) such as acetic, propionic and butyric acids, which are absorbed from the colon. Despite the absence of absorption,

distribution, metabolism and excretion (ADME) data for two modified starches (E 1451 and E 1452) and the absence of in vivo studies in humans for some other modified starches, the Panel considered this database sufficient to conclude that modified starches would not be absorbed intact but significantly hydrolysed by intestinal enzymes and then fermented by intestinal microbiota in humans.”

“In vitro digestibility by pancreatin or saliva was used to compare slightly and highly oxidised cornstarch with unmodified cornstarch and reference starch (unspecified compounds). Maltose production following a fixed interval of enzyme action was taken as a measure of digestibility. The oxidised starch was 10–15% less digestible by pancreatin than unmodified starch, but there was no obvious difference as regards salivary digestion (Documentation provided to EFSA n. 12).

As taken from EFSA, 2017.

4.2. Absorption, distribution and excretion

“Early comparative experiments pointed to inhibition of amylolysis (Tremolières et al., 1959). In vitro digestibility by pancreatin or saliva was used to compare slightly and highly oxidized corn starch with unmodified corn starch and a reference starch. Maltose production after a fixed interval of enzyme action was taken as a measure of digestibility. The oxidized starch was 10-15% less digestible by pancreatin than unmodified starch but there was no obvious difference as regards salivary digestion (Shuman & Mertz, 1959). The digestibility of oxidized wheat starch (conditions not stated) was examined in rats by matched-feeding techniques using the modified starch as the sole source of carbohydrate at a level of 63.7% (dry basis) of the diet. The degree of assimilation by and the general effects on groups of six rats over a feeding period of 28 days were assessed from consideration of body weight changes, faecal residues, digestibility coefficients for starch and postmortem appearance of the animals and their gastrointestinal tracts. The digestibility coefficients were calculated from the starch content of ingested food and residues found in faeces and postmortem gastrointestinal contents. Body weight gain and digestibility coefficients were practically indistinguishable from those obtained for wheat starch or corn starch. Nothing abnormal was noted on postmortem examination (Booher et al., 1951).”

As taken from WHO FOOD ADDITIVES SERIES NO. 5 available at <http://www.inchem.org/documents/jecfa/jecmono/v05je74.htm>

“Other studies, in three groups of three rats each, used corn starch oxidized with 3.9, 4.5, or 5.5% hypochlorite calculated as chlorine. This corresponds to the introduction of 0.57%, (2.04 COOH groups per 100 glucopyranose units), 0.8%, (2.86 COOH groups per 100 glucopyranose units) and 0.9% (3.57 COOH groups per 100 glucopyranose units) carboxyl groups. To 5 g basal diet were added 1, 2 or 4 g modified or control starch and this diet was fed to rats for 10 days. Comparison of digestibilities showed an apparent decrease with increasing oxidation but no effect on caloric values. No tissue damage was associated with the diarrhoea and caecal enlargements observed in groups receiving 2 g or 4 g starch in their feed. Liver, kidney, heart and spleen weights were normal. Diarrhoea and caecal enlargement are known to occur in rats fed starches of poor digestibility or other carbohydrates (White, 1963).” As taken from WHO FOOD ADDITIVES SERIES NO. 5 available at <http://www.inchem.org/documents/jecfa/jecmono/v05je74.htm>

“The digestibility of oxidized starches at levels of 2.5, 6 and 43.2% calculated as chlorine, equivalent to a carboxyl content of 0.32% (1.15 COOH per 100 glucopyranose units), 0.9% (3.81 COOH per 100 glucopyranose units) or 1.46% (5.23 COOH per 100 glucopyranose units), was studied in groups of six male and six female rats. The animals were kept for seven days on 5 g basal diet and then given either 1 g or 2 g starch supplements for 21 days. Poor weight gain with diarrhoea were noted only with the highly oxidized material at both dietary levels. One rat from each of the high dietary level groups was examined. Marked caecal dilatation was seen only in

animals fed the heavily oxidized starch. It is to be noted that this very highly oxidized starch is a commercially unacceptable product

(Whistler & Belfort, 1961).” As taken from WHO FOOD ADDITIVES SERIES NO. 5 available at <http://www.inchem.org/documents/jecfa/jecmono/v05je74.htm>

“The main end products of this colonic anaerobic digestive process are short-chain fatty acids (SCFA) such as acetic, propionic and butyric acids, which are absorbed from the colon. Despite the absence of absorption, distribution, metabolism and excretion (ADME) data for two modified starches (E 1451 and E 1452) and the absence of in vivo studies in humans for some other modified starches, the Panel considered this database sufficient to conclude that modified starches would not be absorbed intact but significantly hydrolysed by intestinal enzymes and then fermented by intestinal microbiota in humans.”

As taken from EFSA, 2017

4.3. Interactions

No data available to us at this time.

5. Toxicity

5.1. Single dose toxicity

“Modified starches (e.g. E 1450) were well tolerated in humans up to a single dose of 25,000 mg/person.”

As taken from EFSA, 2017

5.2. Repeated dose toxicity

“Other studies, in 3 groups of 3 rats each, used corn starch oxidized with 3.9 per cent., 4.5 per cent., or 5.5 per cent. Hypochlorite calculated as chlorine. This corresponds to the introduction of 0.57 per cent., (2.04 COOH groups per 100 glucopyranose units), 0.8 per cent. (2.86 COOH groups per 100 glucopyranose units) and 0.9 per cent. (3.57 COOH groups per 100 glucopyranose units) carboxyl groups. To 5 g basal diet were added 1, 2 or 4 g modified or control starch and this diet was fed to rats for 10 days. Comparison of digestibilities showed an apparent decrease with increasing oxidation but no effect on caloric values. No tissue damage was associated with the diarrhoea and caecal enlargements observed in groups receiving 2 g or 4 g starch in their feed. Liver, kidney, heart and spleen weights were normal. Diarrhoea and caecal enlargement are known to occur in rats fed starches of poor digestibility or other carbohydrates (White, 1963).”

As taken from FAO Nutrition Meetings Report Series 1, available at <http://www.inchem.org/documents/jecfa/jecmono/v001je08.htm>

“The digestibility of oxidized starches at levels of 2.5 per cent., 6 per cent. and 43.2 per cent. calculated as chlorine, equivalent to a carboxyl content of 0.32 per cent. (1.15 COOH per 100 glucopyranose units), 0.9 per cent. (3.81 COOH per 100 glucopyranose units) or 6.4 (?) per cent. (5.23 COOH per 100 glucopyranose units), was studied in groups of 6 male and 6 female rats. The animals were kept for 7 days on 5 g basal diet and then given either 1 g or 2 g starch supplements for 21 days. Poor weight gain with diarrhoea were noted only with the highly oxidized material at all dietary levels. One rat from each of the high dietary level groups showed marked caecal dilation only in animals fed the heavily oxidized starch (Whistler & Belfort, 1961).”

“Starch treated at a level of 0.375 per cent. chlorine was fed to weanling albino rats at 70 per cent.

of their diet for 10 weeks with corn starch as control. Feeding was either unrestricted or by paired-feeding technique. No toxic effects were noted. No details of this work carried out in 1944-5, were available (Garton Sons & Co. Ltd., 1967)."

As taken from FAO Nutrition Meetings Report Series 1, available at <http://www.inchem.org/documents/jecfa/jecmono/v001je08.htm>

"A corn starch oxidized with 5.5% chlorine using sodium hypochlorite (carboxyl content 0.90) was fed to groups of 15 male and 15 female rats at dietary levels of 0, 5, 10 or 25% for 90 days. No adverse effects were noted regarding general health, growth, food intake and efficiency, haematology, serum chemistry and urine analyses. Diarrhoea was not observed. The amount of faeces dry matter/unit food consumed was slightly increased at 25% of the oxidized starch in both sexes. In this group the relative weight of the caecum was slightly increased, the effect being significant in females only. The other organ-to-body weight ratios showed slightly increased adrenal weights of females on the test diets, but the differences with the controls were not dose-related. No other gross changes were observed at autopsy. The histopathological examination has not yet been completed (Til et al., 1973)."

As taken from WHO Food Additives Series 5, available at <http://www.inchem.org/documents/jecfa/jecmono/v05je74.htm>

"A combination of two oral sorbents, oxystarch 35 g/day plus activated charcoal 35 g/day, was administered to four patients undergoing maintenance hemodialysis during thrice weekly and once weekly treatments. Patients tolerated oxystarch and charcoal without complaint during the 4-week period of thrice weekly hemodialyses. All four patients became clinically uremic when hemodialyses were reduced to once weekly and only two patients were able to continue through the end of this 4-week period. Mean serum cholesterol concentration diminished significantly from 200 mg/dl during control periods to 140 mg/dl after each 4-week trial of sorbents (P less than 0.02). Hypertriglyceridemia (range 181 to 543 mg/dl) was corrected in three of four patients with triglyceride values falling to less than 150 mg/dl during ingestion of sorbents (P less than 0.05). Activated charcoal, which is inert as an intestinal nitrogen binding sorbent, may lower serum lipids by direct intragut binding of lipids and bile acids. The potential use of oral charcoal in long-term therapy to reduce hyperlipidemia and prevent vascular accidents due to atherosclerosis requires additional study."

As taken from Friedman EA et al. Am J Clin Nutr. 1978 Oct; 31(10):1903-14. PubMed, 2010 available at <http://www.inchem.org/documents/jecfa/jecmono/v05je74.htm>

"No treatment-related effects relevant for human risk assessment were observed in rats fed very high levels of modified starches (up to 31,000 mg/kg body weight (bw) per day)."

"The digestibility of a great number of unmodified or modified starches was compared in rats (Booher et al., 1951). Among them, the digestibility of a commercial wheat preparation oxidised with hypochlorite, a 'thin-boiling' product, was tested in weanling male rats (n = 6, body weight 45–60 g, strain not specified) over a feeding period of 28 days. Matched-feeding techniques were used, with the modified starch as the sole source of carbohydrate at a level of 63.7% (on dry basis) of the diet. Body weight changes, faecal residues and digestibility coefficients for each starch were investigated. After sacrifice, changes were noted in the gastrointestinal tracts of the animals. The digestibility coefficients were calculated from the starch content of ingested food, residues found in faeces and post-mortem gastrointestinal contents. There were no significant differences in body weight gain and digestibility coefficients between the oxidised and the corresponding control wheat starch. Post-mortem examination showed no differences."

“Cornstarch oxidised with 2.5%, 6% and 43.2% hypochlorite calculated as chlorine (= carboxyl groups introduced: 0.32%; 1.15 COOH per 100 glucopyranose units, 0.9%; 3.81 COOH per 100 glucopyranose units, 1.46%; 5.23 COOH per 100 glucopyranose units) was fed to rats (6 animals per sex and group, Wistar–Purdue strain) (Whistler and Belfort, 1961). The animals were kept for 7 days on 5 g basal diet and then given either 1 or 2 g starch supplements for 21 days. The weight gain was unaffected. Poor weight gain with diarrhoea was noted only with the highly oxidised material (43.2%) at both dietary levels. One rat from each of the high dietary level groups was examined. Marked caecal dilation was seen only in animals fed the highly oxidised starch. According to the authors, this very highly oxidised non-commercial starch was prepared only to determine whether any effect on weight gain could be produced by feeding an overoxidised product.”

“Cornstarch oxidised with 3.9%, 4.5% or 5.5% hypochlorite calculated as chlorine (= carboxyl groups introduced: 0.57%; 2.04 COOH groups per 100 glucopyranose units, 0.8%; 2.86 COOH groups per 100 glucopyranose units, 0.9%; 3.57 COOH groups per 100 glucopyranose units) was fed to rats (3 animals per group, strain and sex not specified) for 10 days (White, 1963). One, 2 or 4 g modified or control starch were added to 5 g basal diet. With increasing oxidation, digestibility slightly decreased, but no effect on caloric values was observed. No tissue damage was associated with the diarrhoea and caecal enlargements observed in the groups receiving 2 or 4 g starch. Liver, kidney, heart and spleen weights were normal. Diarrhoea and caecal enlargement are known to occur in rats fed starches of poor digestibility or other carbohydrates.”

“Starch treated at a level of 0.375% with chlorine was fed to weanling albino rats at 70% of their diet (equal to 63,000 mg/kg bw per day) for 10 weeks, using cornstarch as the control (Garton & Sons Co. Ltd., 1967; cited in JECFA, 1975a). Feeding was either unrestricted or followed the paired-feeding technique. No toxic effects were noted. No details of this work, carried out in 1944–1945, were available.”

“An oxidised starch, obtained by treating cornstarch with 5.5% chlorine, using sodium hypochlorite (carboxyl content 0.90), was fed to groups of 15 male and 15 female weanling albino Wistar rats at dietary levels of 0.5%, 10% or 25% for 90 days (equal to 450, 9,000 and 22,500 mg/kg bw per day) (Documentation provided to EFSA n. 20). General condition, behaviour and survival were not adversely affected at any dietary level of the modified starch. Growth and food intake showed no distinct differences between the test groups and the controls in either sex. The water content of the faeces was comparable in all groups. Gross and microscopic pathological examination showed no treatment-related pathological changes. Diarrhoea was not observed, but the production of faeces dry matter at 25% of the oxidised starch-treated animals was increased compared to the controls. This phenomenon was accompanied by an increase in the weight of the caecum, both filled and empty, at the 25% level, in females only. However, no histological changes could be detected in this organ. Haematological indices, biochemical blood values and urine composition showed no treatment-related differences. From the results of this study it was concluded that feeding of the modified starch at levels up to 25% in the diet in rats did not induce any distinct adverse effects.”

As taken from EFSA, 2017

5.3. Reproduction toxicity

No data available to us at this time.

5.4. Mutagenicity

“Evaluation of genotoxicity of the modified starches evaluated in the present opinion was performed *in silico*, since no genotoxicity studies were available. The Panel concluded that the *in silico* analyses of the substructures of modified starch moieties did not identify any relevant alert for genotoxicity, and concluded that modified starches do not raise concern for genotoxicity.”

As taken from EFSA, 2017

5.5. Cytotoxicity

No data available to us at this time.

5.6. Carcinogenicity

No data available to us at this time.

5.7. Irritation/immunotoxicity

No data available to us at this time.

5.8. All other relevant types of toxicity

“A USA Food and Drug select committee on Generally Recognized As Safe (GRAS) substances (US FDA, 2016) indicated that the digestibility and caloric value of starch oxidized with the maximum permitted level of sodium hypochlorite, as determined in rat feeding tests, were similar to those for unmodified starch (Til and Kuper, 1993). However, starch treated with about eight times the permitted level of hypochlorite caused diarrhea and marked growth depression and cercal enlargement in rats in 21day feeding studies, demonstrating the adverse effects of starches containing high levels of oxidized groups. High dose groups fed 30% oxidized starch also noted treatment related changes in the kidney and urothelial epithelium associated with mineralization (Til and Kuper, 1993). No specific toxicity was noted in other organs or systems and the primary effects were limited to the gastrointestinal system. When oxidizing starch according to manufacturing guidelines and used at the permitted concentrations, the toxicological profile for oxidized starch is not expected to be significantly different than that of native starch.”

As taken from ECCC, 2018

6. Functional effects on

6.1. Broncho/pulmonary system

No data available to us at this time.

6.2. Cardiovascular system

No data available to us at this time.

6.3. Nervous system

No data available to us at this time.

6.4. Other organ systems, dependent on the properties of the substance

“With the purpose of screening potential antimalarial agents, oxidized starch imine derivatives of sulfonamides or pyrimidine - derivatives of sulfoxazole (ML8), sulfameter (ML11) and trimethoprim (ML13) - and oxidized cellulose imine derivatives of dapsone (ML14), sulfadiazine (ML17), sulfamethoxazole (ML18), sulfoxazole (ML19), sulfamethoxypyridazine (ML20) and sulfameter (ML22) were submitted to in vivo biological assays with mice infected with *Plasmodium berghei*. Only ML11 was 100% curative in test conditions; ML 17 showed the same effect as its prototype.

The drug content in both prodrugs is lower in the applied dose than that used in the original drugs. It can be suggested that the latentiation enhanced the effectiveness of the prototypes.”

As taken from Ohara MT et al. Boll Chim Farm. 1995 Oct; 134(9):522-7. PubMed, 2010 available at <http://www.ncbi.nlm.nih.gov/pubmed/8527105?dopt=AbstractPlus>

7. Addiction

JTI is not aware of any information that demonstrates that this ingredient has any addictive effect.

8. Burnt ingredient toxicity

In comparison with a CSC of a reference cigarette with sideseam adhesives/cigarette paper corresponding to representative specifications for the majority of commercial cigarettes no differences were observed either in the bacterial mutagenicity, cytotoxicity or mammalian cell genotoxicity of the smoke condensate prepared from cigarettes with sideseam adhesives/cigarette paper containing Starch, Food, Modified: Oxidized Starches at 2.0869 mg/cig. The smoke chemistry data between test and reference cigarette revealed small changes towards both higher and lower yields per cigarette over all analytical groups. These differences were well within the variability of the analytical methods (JTI NTM Study Report(s)).

9. Heated/vapor emissions toxicity

Aerosol from heated tobacco stick(s) containing Starch and/or Modified starches was tested in aerosol chemistry and a battery of in vitro test(s). Under the test conditions and within the sensitivity and specificity of the bioassay(s), the activity of the total particulate matter (TPM) and/or gas vapor phase (GVP) were not increased by the addition of this ingredient when compared to TPM and/or GVP from reference combustible cigarettes. The table below provides the highest tested level(s) and specific endpoint(s):

Endpoint	Tested level (mg/stick)	Reference
Aerosol chemistry	1.48	Labstat International Inc. (2020a) Labstat International Inc. (2021a)
In vitro genotoxicity	1.48	Labstat International Inc. (2020b) Labstat International Inc. (2021b)
In vitro cytotoxicity	1.48	Labstat International Inc. (2020b) Labstat International Inc. (2021b)

10. Ecotoxicity

10.1. Environmental fate

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that oxidized starch (CAS RN 65996-62-5) is of uncertain persistence in the environment.

Data accessed December 2014 on the OECD website:
<http://webnet.oecd.org/CCRWeb/Search.aspx>

10.2. Aquatic toxicity

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that oxidized starch (CAS RN 65996-62-5) is not inherently toxic to aquatic organisms and is of low ecotoxicological concern.

Data accessed December 2014 on the OECD website:
<http://webnet.oecd.org/CCRWeb/Search.aspx>

“Deicers currently used for aircraft deicing, including ethylene glycol and propylene glycol, pose significant threats to surface waters, as a result of high biochemical oxygen demand (BOD) and toxicity to aquatic organisms. Oxidized starch may provide a less toxic deicer with lower BOD. The freezing point depression of starch formulations oxidized using hydrogen peroxide and catalysts (i.e., catalyzed hydrogen peroxide [H₂O₂] propagations-CHP) was 28 degrees C, and viscosities similar to those of commercial deicers were achieved after post-treatment with granular activated carbon. The most effective oxidized starch formulation exerted a 5-day BOD up to 6 times lower than glycol deicers (103 versus 400 to 800 g O₂/L). Toxicity to *Ceriodaphnia dubia* for this formulation (48-hour lethal concentration, 50% [LC₅₀] of 2.73 g/L) was greater than pure propylene glycol (13.1 g/L), but lower than propylene glycol deicer formulations (1.02 g/L). Organic acids were identified by gas chromatography/mass spectrometry as the primary constituents in the oxidized starch solution. The proposed deicing system would provide effective deicing while exerting minimal environmental effects (e.g., lower toxicity to aquatic organisms and lower BOD)...” As taken from Plahuta JM et al. 2011. *Water Environ. Res.* 83(9), 826-33. PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/22073730?dopt=AbstractPlus>

10.3. Sediment toxicity

No data available to us at this time.

10.4. Terrestrial toxicity

No data available to us at this time.

10.5. All other relevant types of ecotoxicity

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that oxidized starch (CAS RN 65996-62-5) is of uncertain bioaccumulative potential in the environment.

Data accessed December 2014 on the OECD website:
<http://webnet.oecd.org/CCRWeb/Search.aspx>

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12. Other information

No data available to us at this time.

13. Last audited

June 2023

INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

WORLD HEALTH ORGANIZATION

**TOXICOLOGICAL EVALUATION OF SOME
FOOD COLOURS, ENZYMES, FLAVOUR
ENHANCERS, THICKENING AGENTS, AND
CERTAIN FOOD ADDITIVES**

WHO FOOD ADDITIVES SERIES 6

The evaluations contained in this publication were prepared by the
Joint FAO/WHO Expert Committee on Food Additives which met in Rome,
4-13 June 1974¹

World Health Organization Geneva 1975

¹ Eighteenth Report of the Joint FAO/WHO Expert Committee on
Food Additives, Wld Hlth Org. techn. Rep. Ser., 1974, No. 557.
FAO Nutrition Meetings Report Series, 1974, No. 54.

OXIDIZED STARCHES*

Explanation

These compounds have been evaluated for acceptable daily intake
by the Joint FAO/WHO Expert Committee on Food Additives (see Annex 1,

Refs Nos 20, 27 and 34) in 1969, 1971 and 1973.

Since the previous evaluation additional data have become available and are summarized and discussed in the following monograph. The previously published monographs have been expanded and are reproduced in their entirety below.

BIOLOGICAL DATA

BIOCHEMICAL ASPECTS

Early comparative experiments pointed to inhibition of amylolysis (Tremolieres et al., 1959). In vitro digestibility by pancreatin or saliva was used to compare slightly and highly oxidized corn starch with unmodified corn starch and reference starch. Maltose production after a fixed interval of enzyme action was taken as a measure of digestibility. The oxidized starch was 10-15% less digestible by pancreatin than unmodified starch but there was no obvious difference as regards salivary digestion (Shuman, C. C. & Mertz, E. T., 1959). The digestibility of oxidized wheat starch (conditions not stated) was examined in rats by matched-feeding techniques using the modified starch as the sole source of carbohydrate at a level of 63.7% (dry basis) of the diet. The degree of assimilation by and the general effects on groups of six rats over a feeding period of 28 days were assessed from consideration of body weight changes, faecal residues, digestibility coefficients for starch and post-mortem appearance of the animals and their gastrointestinal tracts. The digestibility coefficients were calculated from the starch content of ingested food and residues found in faeces and post-mortem gastrointestinal contents. Body weight gain and digestibility coefficients were practically indistinguishable from those obtained for wheat starch or corn starch. Nothing abnormal was noted on post-mortem examination (Booher et al., 1959).

* Whatever oxidizing agent is used only minor modifications of the starch molecule are achieved in normal manufacturing practice. These are equivalent to the introduction of 1% w/w of carboxyl (-COOH) or 0.5% w/w of keto (-CO) groups, or 3.6 carboxyl and 2.9 carbonyl groups per 100 glucopyranose units. No chlorine is introduced into the molecule and the final products usually contain only residues of sodium chloride, sodium sulfate and sulfur dioxide.

Other studies, in three groups of three rats each, used corn starch oxidized with 3.9%, 4.5% or 5.5% hypochlorite calculated as chlorine. This corresponds to the introduction of 0.57% (2.04 COOH groups per 100 glucopyranose units), 0.8% (2.86 COOH groups per 100

glucopyranose units) and 0.9% (3.57 COOH groups per 100 glucopyranose units) carboxyl groups. To 5 g basal diet were added 1, 2 or 4 g modified or control starch and this diet was fed to rats for 10 days. Comparison of digestibilities showed an apparent decrease with increasing oxidation but no effect on caloric values. No tissue damage was associated with the diarrhoea and caecal enlargements observed in groups receiving 2 g or 4 g starch in their feed. Liver, kidney, heart and spleen weights were normal. Diarrhoea and caecal enlargement are known to occur in rats fed starches of poor digestibility or other carbohydrates (White, 1963).

The digestibility of oxidized starches at levels of 2.5%, 6% and 43.2% calculated as chlorine, equivalent to a carboxyl content of 0.32% (1.15 COOH per 100 glucopyranose units), 0.9% (3.81 COOH per 100 glucopyranose units) or 1.46% (5.23 COOH per 100 glucopyranose units) was studied in groups of six male and six female rats. The animals were kept for seven days on 5 g basal diet and then given either 1 g or 2 g starch supplements for 21 days. Poor weight gain with diarrhoea were noted only with the highly oxidized material at both dietary levels. One rat from each of the high dietary level groups was examined. Marked caecal dilation was seen only in animals fed the heavily oxidized starch. It is to be noted that this very highly oxidized starch is a commercially unacceptable product (Whistler & Belfort, 1961).

TOXICOLOGICAL STUDIES

Short-term studies

Rat

Starch treated at a level of 0.375% chlorine was fed to weanling albino rats at 70% of their diet for 10 weeks with corn starch as control. Feeding was either unrestricted or by paired-feeding technique. No toxic effects were noted. No details of this work, carried out in 1944-1945, were available (Garton Sons & Co. Ltd, 1967).

A corn starch oxidized with 5.5% chlorine using sodium hypochlorite (carboxyl content 0.90) was fed to groups of 15 male and 15 female rats at dietary levels of 0.5, 10 or 25% for 90 days. No adverse effects were noted regarding general health, growth, food intake and efficiency, haematology, serum chemistry and urine analyses. Diarrhoea was not observed. The amount of faeces dry matter/unit food consumed was slightly increased at 25% of the

oxidized starch in both sexes. In this group the relative weight of the caecum was slightly increased, the effect being significant in

females only. The other organ-to-body weight ratios showed slightly increased adrenal weights of females on the test diets, but the differences with the controls were not dose-related. No other gross changes were observed at autopsy. No compound related effects were observed. Haematologic studies at week 13 showed no distinct or significant differences between tests and control groups (Til et al., 1973; Til et al., 1974).

Long-term studies

None supplied.

Comments:

The digestibility of hypochlorite-oxidized starch has been investigated in vivo and found to be similar to that of unmodified starch. The longest of the short-term studies extends over 90 days and showed no serious toxic effects at the 25% dietary level, the highest level-tested. The full results of this study have now been reported. Studies with highly oxidized starches (14% or more carboxyl group) are not applicable because these products are not useful as food additives. Provided the chemical change is limited to the introduction of no more than one carboxyl group per 25 anhydroglucose units, the biological effects of the modified starch do not appear to be deleterious. The requirements of the Committee have now been met.

EVALUATION

Acceptable daily intake not specified.*

* The statement "ADI not specified" means that, on the basis of the available data (toxicological, biochemical, and other), the total daily intake of the substance, arising from its use or uses at the levels necessary to achieve the desired effect and from its acceptable background in food, does not, in the opinion of the Committee, represent a hazard to health. For this reason, and for the reasons, stated in individual evaluations, the establishment of any acceptable daily intake (ADI) in mg per kg of body weight is not deemed necessary.

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See Also:

Toxicological Abbreviations

Oxidized starches (FAO Nutrition Meetings Report Series 46a)

Oxidized starches (WHO Food Additives Series 1)

Oxidized starches (WHO Food Additives Series 5)

ADOPTED: 22 June 2017

AMENDED: 31 October 2017

doi: 10.2903/j.efsa.2017.4911

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 Panel on Food Additives and Nutrient Sources added to Food (ANS),
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Birgit Dusemund, Maria Jose Frutos, Pierre Galtier, David Gott, Ursula Gundert-Remy,
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Dominique Parent-Massin, Agneta Oskarsson, Ivan Stankovic, Ine Waalkens-Berendsen,
Matthew Wright, Maged Younes, Paul Tobback, Zsuzsanna Horvath, Stavroula Tasiopoulou
and Rudolf Antonius Woutersen

Abstract

Following a request from the European Commission, the EFSA Panel on Food Additives and Nutrient sources added to Food (ANS) was asked to deliver a scientific opinion on the re-evaluation of 12 modified starches (E 1404, E 1410, E 1412, E 1413, E 1414, E 1420, E 1422, E 1440, E 1442, E 1450, E 1451 and E 1452) authorised as food additives in the EU in accordance with Regulation (EC) No 1333/2008 and previously evaluated by JECFA and the SCF. Both committees allocated an acceptable daily intake (ADI) 'not specified'. In humans, modified starches are not absorbed intact but significantly hydrolysed by intestinal enzymes and then fermented by the intestinal microbiota. Using the read-across approach, the Panel considered that adequate data on short- and long-term toxicity and carcinogenicity, and reproductive toxicity are available. Based on *in silico* analyses, modified starches are considered not to be of genotoxic concern. No treatment-related effects relevant for human risk assessment were observed in rats fed very high levels of modified starches (up to 31,000 mg/kg body weight (bw) per day). Modified starches (e.g. E 1450) were well tolerated in humans up to a single dose of 25,000 mg/person. Following the conceptual framework for the risk assessment of certain food additives, the Panel concluded that there is no safety concern for the use of modified starches as food additives at the reported uses and use levels for the general population and that there is no need for a numerical ADI. The combined exposure to E 1404–E 1451 at the 95th percentile of the refined (brand-loyal) exposure assessment scenario for the general population was up to 3,053 mg/kg bw per day. Exposure to E 1452 for food supplement consumers only at the 95th percentile was up to 22.1 mg/kg bw per day.

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Keywords: modified starches, E 1404, E 1410, E 1412, E 1413, E 1414, E 1420, E 1422, E 1440, E 1442, E 1450, E 1451, E 1452

Requestor: European Commission

Question numbers: EFSA-Q-2011-00489, EFSA-Q-2011-00490, EFSA-Q-2011-00491, EFSA-Q-2011-00492, EFSA-Q-2011-00493, EFSA-Q-2011-00494, EFSA-Q-2011-00495, EFSA-Q-2011-00496, EFSA-Q-2011-00497, EFSA-Q-2011-00498, EFSA-Q-2011-00499, EFSA-Q-2013-00696

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Acknowledgements: The Panel wishes to thank Jacqueline Wiesner for the support provided to this scientific output. The ANS Panel wishes to acknowledge all European competent institutions, Member State bodies and other organisations that provided data for this scientific output.

Amendment: The updated version of this scientific opinion includes minor changes of an editorial nature that do not materially affect the contents. To avoid confusion, the original version of the scientific opinion has been removed from the website, but is available on request, as is a version showing all the changes made.

Suggested citation: EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), Mortensen A, Aguilar F, Crebelli R, Di Domenico A, Dusemund B, Frutos MJ, Galtier P, Gott D, Gundert-Remy U, Lambré C, Leblanc J-C, Lindtner O, Moldeus P, Mosesso P, Parent-Massin D, Oskarsson A, Stankovic I, Waalkens-Berendsen I, Wright M, Younes M, Tobback P, Horvath Z, Tasiopoulou S and Woutersen RA, 2017. 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 Journal 2017;15(10):4911, 96 pp. <https://doi.org/10.2903/j.efsa.2017.4911>

ISSN: 1831-4732

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Figure 3 and Figure 4: CCR Press



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Summary

Following a request from the European Commission, the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to deliver a scientific opinion re-evaluating the safety 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 starch phosphate (E 1442), starch sodium octenyl succinate (E 1450), acetylated oxidised starch (E 1451) and starch aluminium octenyl succinate (E 1452) when used as food additives.

The aforementioned modified starches are authorised as food additives in accordance with Regulation (EC) No 1333/2008.

Starch typically consists of two polymers of glucose, namely amylose, with an almost linear structure, and amylopectin, which is highly branched. In amylose, the glucose monomers (pyranosic form) are linked by α -1,4-glycosidic links, while amylopectin contains additionally α -1,6-glycosidic bonds. Commercial starches are composed of about 20–25% amylose and 75–80% amylopectin. High amylose starches typically consist of 50–80% amylose and 20–50% amylopectin. Starches for commercial use are generally produced from potatoes, cereals or other sources.

In modified starches, the chemical and physical characteristics of the native substances are altered in order to improve the functional properties for particular food applications: the observed effects on such properties depend on the type and extent of the modification (e.g. degree of substitution (DS)) and the source starch (e.g. cereal, potato, tapioca). In general, the extent of modification required to distinctly alter the functional characteristics of native starches is low, as imposed by Commission Regulation (EU) No 231/2012. In conclusion, for a large number of food applications, modified starches are used because of their superior properties compared to the native substances.

Modified starches have been previously evaluated by the Scientific Committee for Food (1976, 1981, 1990 and 1995) and by the Joint FAO/WHO Expert Committee on Food Additives (1969, 1971, 1973, 1982, 2014 and 2016). An acceptable daily intake (ADI) 'not specified' was allocated by both committees.

Data on *in vitro* degradation of modified starches by digestive enzymes indicated that their digestibility was slightly reduced or showed no differences when compared to corresponding unmodified starches. *In vivo* data are in agreement with *in vitro* studies indicating that the two major components of starches, amylose and amylopectin, would be fermented during their passage through the large intestine by strains of bacteria found in the human colon. The main end products of this colonic anaerobic digestive process are short-chain fatty acids (SCFA) such as acetic, propionic and butyric acids, which are absorbed from the colon. Despite the absence of absorption, distribution, metabolism and excretion (ADME) data for two modified starches (E 1451 and E 1452) and the absence of *in vivo* studies in humans for some other modified starches, the Panel considered this database sufficient to conclude that modified starches would not be absorbed intact but significantly hydrolysed by intestinal enzymes and then fermented by intestinal microbiota in humans.

Toxicity data were not available for all of the modified starches evaluated in the present opinion and for all endpoints. In general, the most complete data sets were available for acetylated distarch phosphate (E 1414) and acetylated distarch adipate (E 1422). However, given their structural, physicochemical and biological similarities, it is possible to read-across between all the modified starches.

Data concerning acute oral toxicity are available for several animal species for distarch phosphate (E 1412) only. These indicate low oral acute toxicity.

Short-term and/or subchronic (90-day) studies were available in rats for all modified starches, except monostarch phosphate (E 1410). Occasionally also, studies in dogs, pigs or hamsters were available. The modified starches were given at dietary levels up to 70%. The test duration was up to 90 days. Effects on body weight and feed consumption were not observed up to dietary levels of 25%. Caecum weight was increased at exposure levels of 30% and higher, but histopathological changes were not observed. The only frequently observed significant histopathological change was the presence of pelvic and/or corticomedullary mineralisation in the kidneys, which was observed with modified as well as unmodified starches, and occurred more pronounced in females than in males.

In a 90-day study with acetylated oxidised starch (E 1451) in rats, a no-observed-adverse-effect level (NOAEL) of 10% in the diet, equal to 5,900 mg/kg per day, was determined based on hyperplasia of the transitional epithelium of the urinary bladder and the kidneys.

Evaluation of genotoxicity of the modified starches evaluated in the present opinion was performed *in silico*, since no genotoxicity studies were available. The Panel concluded that the *in silico* analyses of the substructures of modified starch moieties did not identify any relevant alert for genotoxicity, and concluded that modified starches do not raise concern for genotoxicity.

Two chronic (52-week) studies were available, one with acetylated distarch phosphate (E 1414) and one with acetylated distarch adipate (E 1422). At necropsy, relative organ weights showed no differences between the groups, except for caecal enlargement. Histopathological examination of kidneys demonstrated the presence of treatment-related pelvic nephrocalcinosis. A clear correlation was observed between the increased incidence of pelvic nephrocalcinosis, increased accumulation of calcium in the kidneys and increased urinary excretion of calcium.

Carcinogenicity studies were available for E 1413, E 1414, E 1420, E 1422, E 1442 and E 1450 in rats and for E 1442 in mice. There was no evidence for carcinogenicity. The long-term studies in rats did not reveal any significant effect, except for caecal enlargement. As this effect was observed without associated histopathological changes, it was considered to be of no toxicological significance for humans.

Kidney lesions (pelvic and corticomedullary mineralisation) occurred in rats (sub)chronically fed high levels (up to 62% in the diet, equivalent to 31,000 mg/kg body weight (bw) per day) of phosphated distarch phosphate (E 1413), acetylated distarch phosphate (E 1414), acetylated starch (E 1420), acetylated distarch adipate (E 1422) and hydroxypropyl distarch phosphate (E 1442). The lesions were considered to be associated with an imbalance of Ca/P and Mg in the diet. The mechanism is considered to be related to increased calcium absorption in the lower intestine caused by the formation of absorbable breakdown products. As the rat is a particularly sensitive species for pelvic nephrocalcinosis, while these lesions were not observed in the hamster and the pig, it was considered to be of no relevance for risk assessment in humans.

Dietary reproductive toxicity studies in rats were available for phosphate distarch phosphate (E 1413), acetylated distarch phosphate (E 1414), acetylated starch (E 1420) and acetylated distarch adipate (E 1422). No effects on reproductive performance or maternal and developmental effects were observed in the three-generation reproductive toxicity studies at dietary levels of up to 62% (equivalent to 31,000 mg/kg bw per day).

No developmental toxicity studies were available.

Studies in healthy human volunteers with phosphated distarch phosphate (E 1413), acetylated distarch phosphate (E 1414) and acetylated starch (E 1420) reported no adverse effects at doses of 60,000 mg/person.

Starch sodium octenyl succinate (E 1450) up to a single dose of 25,000 mg was well tolerated by fasting healthy adults. However, the Panel noted reports on gastrointestinal symptoms conducted in infants with hypoallergenic formula containing 2% octenyl succinic anhydride (OSA)-modified starch (24,000 mg/person).

To assess the dietary exposure to modified starches (E 1404–E 1451) from their use as food additives, the combined exposure was calculated based on (1) maximum reported use levels provided to EFSA (defined as the *maximum level exposure assessment scenario*), and (2) reported use levels (defined as the *refined exposure assessment scenario*, brand-loyal and non-brand-loyal consumer scenario).

Modified starches are authorised in a wide range of foods. The Panel did identify brand loyalty to specific food categories in infants and toddlers (e.g. processed cereal baby foods, unflavoured fermented milk products and flavoured fermented milk products). Further, the Panel considered that the non-brand-loyal scenario covering other population groups was appropriate and realistic scenario for risk characterisation because it is assumed that the population would probably be exposed long-term to the food additive present at the mean reported use in processed food.

A refined estimated exposure assessment scenario taking into account the food for special medical purposes (FSMP) for infants and young children (FC 13.1.5.1 Dietary foods for infants for special medical purposes and special formulae for infants and FC 13.1.5.2 Dietary foods for babies and young children for special medical purposes as defined by Commission Directive 1999/22/EC) was also performed to estimate exposure of infants and toddlers who may be on a specific diet. Considering that this diet is required due to specific needs, it is assumed that consumers are loyal to the food brand; therefore, the refined brand-loyal estimated exposure scenario was performed.

A specific *food supplement consumers only* scenario was also performed to estimate exposure for children, adolescents, adults and the elderly, as exposure via food supplements may deviate largely

from that via food, and the number of food supplement consumers may be low depending on populations and surveys.

The refined estimates were based on 36 out of 72 food categories in which modified starches are authorised. The Panel considered that the uncertainties identified would, in general, result in an overestimation of the exposure to modified starches as a food additive in European countries for the maximum level exposure scenario. However, the Panel noted that given the information from the Mintel's Global New Products Database (GNPD), it may be assumed that modified starches are used in food categories ($n = 13$) for which no data have been provided by food industry. The main food categories, in terms of amount consumed, not taken into account were processed fish and fishery products, including molluscs and crustaceans, breakfast cereals, salads and savoury-based sandwich spreads. According to the Mintel GNPD, in the European Union (EU) market, these categories are labelled with modified starches. Therefore, the Panel considered that if these uncertainties were confirmed, it would therefore result in an underestimation of the exposure.

The Panel further noted that the exposure to modified starches (E 1404–E 1451) from their use according Annex III to Regulation (EC) No 1333/2008 (Parts 1, 3 and 5) was not considered in the exposure assessment.

Separate scenarios were carried out for the exposure assessment of starch aluminium octenyl succinate (E 1452), taking into account the consumption of food supplements for consumers only and based on the maximum permitted level (MPL) (*regulatory maximum exposure assessment scenario*) and on the maximum reported use level (*maximum reported level exposure assessment scenario*). Exposure to aluminium from the use of E 1452 as a food additive was also estimated.

Exposure to aluminium from the use of E 1452 in the *regulatory maximum level exposure assessment scenario* ranged for all population groups from 0.8% to 26% of the tolerable weekly intake (TWI) of 1 mg aluminium/kg bw established by EFSA (2008) at the mean, and up to 47% at the 95th percentile. For the *maximum reported level exposure assessment scenario*, based on the usage levels provided by food industry, exposure to aluminium from E 1452 ranged from < 0.1 at the mean, up to 2.5% for the 95th percentile across population groups. Furthermore, according to the information provided by industry, the content of aluminium in E 1452 is significantly lower than the limit set in the EU specifications for E 1452.

The Panel also noted that the refined exposure estimates are based on information provided on the reported levels of use of modified starches. If actual practice changes, this refined estimates may no longer be representative and should be updated.

Following the conceptual framework for the risk assessment of certain food additives re-evaluated under Commission Regulation (EU) No 257/2010 (EFSA ANS Panel, 2014) and given that:

- adequate combined exposure data were available; in the general population, the 95th percentile of the refined exposure, calculated based on the use levels reported from food industry, was up to 3,053 mg/kg bw per day for toddlers (brand-loyal consumer scenario);
- an indicative refined exposure to modified starches (E 1404–E 1451) of up to 991 mg/kg bw per day has been calculated at the 95th percentile for children, for the population consuming food supplements;
- exposure to starch aluminium octenyl succinate (E 1452) for food supplement consumers only at the 95th percentile was 22.1 mg/kg bw per day (*regulatory maximum level exposure assessment scenario*) and 1.2 mg/kg bw per day (*maximum reported level exposure assessment scenario*) in the elderly;
- their structural, physicochemical and biological similarities, allow for read-across between all the modified starches;
- the ADME database is sufficient to conclude that, in humans, modified starches would not be absorbed intact, but significantly hydrolysed by intestinal enzymes and then fermented by the intestinal microbiota;
- using the read-across approach, adequate data on short- and long-term toxicity and carcinogenicity and reproductive toxicity are available;
- no treatment-related effects relevant for human risk assessment were observed in long-term studies in rats fed very high levels of modified starches (up to 31,000 mg/kg bw per day);
- although no genotoxicity data on the modified starches evaluated in the present opinion were available, modified starches are not of genotoxic concern based on *in silico* analysis;
- modified starches (i.e. E 1413, E 1414, E 1420 and E 1450) were well tolerated in adults up to a single daily dose of 60,000 mg/person (860 mg/kg bw);

the Panel concluded that there is no safety concern for the use of modified starches as food additives at the reported uses and use levels and that there is no need for a numerical ADI.

Concerning the use of starch sodium octenyl succinate (E 1450) in 'dietary foods for special medical purposes and special formulae for infants' (food category 13.1.5.1) and of E 1404, E 1410, E 1412, E 1413, E 1414, E 1420, E 1450 and E 1451 in food belonging to food category 13.1.5.2 and given that:

- for populations consuming foods for special medical purposes and special formulae, the 95th percentile of exposure calculated based on the maximum use levels reported from food industry was up to 5,286 mg/kg bw per day for infants;
- infants and young children consuming foods belonging to these food categories may show a higher susceptibility to the gastrointestinal effects of modified starches than their healthy counterparts due to their underlying medical condition;
- no effects on body weight and food intake were observed in male and female neonatal pigs exposed to 10,000 mg/kg bw per day of OSA-modified starch (E 1450) in formula for 21 days;
- OSA-modified starch (E 1450), up to a single dose of 25,000 mg/person, was well tolerated by fasting healthy adults, but gastrointestinal symptoms were reported in infants with hypoallergenic formula containing 2% of OSA-modified starch (about 24,000 mg/person);
- available information on the clinical studies in infants is limited and results refer to the feeding of formula containing OSA-modified starch in concentrations below 2%, the current authorised MPL,

the Panel concluded, that the available data do not allow for an adequate assessment of the safety of the use of starch sodium octenyl succinate (E 1450) in 'dietary foods for special medical purposes and special formulae for infants' (food category 13.1.5.1) or of E 1404, E 1410, E 1412, E 1413, E 1414, E 1420, E 1450 and E 1451 in foods belonging to food category 13.1.5.2, in infants and young children consuming these foods at the presently authorised maximum use levels of 20,000 or 50,000 mg/kg, respectively.

The Panel recommended that:

- the European Commission considers revising the maximum limits for the toxic elements arsenic, lead and mercury present as impurities in the EU specifications for all modified starches re-evaluated in the present opinion (E 1404, E 1410, E 1412, E 1413, E 1414, E 1420, E 1422, E 1440, E 1442, E 1450, E 1451 and E 1452) to ensure that these food additives will not be a significant source of exposure to these toxic elements in food;
- the European Commission considers revising specifications, including harmonisation of microbiological criteria for polysaccharides such as modified starches and gums, and taking into account future availability of specific methods of analysis of modified starches;
- the European Commission seeks confirmation on the actual use of starch aluminium octenyl succinate (E 1452) in its currently permitted use limited to food supplements (only vitamin preparations for encapsulation purposes);
- additional data should be generated to assess the potential health effects of starch sodium octenyl succinate (E 1450) when used in 'dietary foods for special medical purposes and special formulae for infants' (food category 13.1.5.1) or of E 1404, E 1410, E 1412, E 1413, E 1414, E 1420, E 1450 and E 1451 in foods belonging to food category 13.1.5.2;
- due to the discrepancies observed between the data reported from industry and the Mintel database, where modified starches (E 1404–E 1451) are labelled in more products than in food categories for which data were reported from industry, the Panel recommended collection of data on usage and use levels of modified starches (E 1404–E 1451) in order to perform a more realistic exposure assessment.

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1. Introduction

The present opinion deals with the re-evaluation of the safety 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) when used as food additives. These modified starches are authorised food additives in the EU according to Annex II and Annex III to Regulation (EC) No 1333/2008¹.

1.1. Background and Terms of Reference as provided by the European Commission

1.1.1. Background

Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives requires that food additives are subject to a safety evaluation by the European Food Safety Authority (EFSA) before they are permitted for use in the European Union (EU). In addition, it is foreseen that food additives must be kept under continuous observation and must be re-evaluated by EFSA.

For this purpose, a programme for the re-evaluation of food additives that were already permitted in the European Union before 20 January 2009 has been set up under Regulation (EU) No 257/2010². This Regulation also foresees that food additives are re-evaluated whenever necessary in light of changing conditions of use and new scientific information. For efficiency and practical purposes, the re-evaluation should, as far as possible, be conducted by group of food additives according to the main functional class to which they belong.

The order of priorities for the re-evaluation of the currently approved food additives should be set on the basis of the following criteria: the time since the last evaluation of a food additive by the Scientific Committee on Food (SCF) or by EFSA, the availability of new scientific evidence, the extent of use of a food additive in food and the human exposure to the food additive taking also into account the outcome of the Report from the Commission on Dietary Food Additive Intake in the EU³ of 2001. The report 'Food additives in Europe 2000' submitted by the Nordic Council of Ministers to the Commission, provides additional information for the prioritisation of additives for re-evaluation.

In 2003, the Commission already requested EFSA to start a systematic re-evaluation of authorised food additives. However, as a result of adoption of Regulation (EU) 257/2010, the 2003 Terms of References are replaced by those below.

1.1.2. Terms of Reference

The Commission asks EFSA to re-evaluate the safety of food additives already permitted in the Union before 2009 and to issue scientific opinions on these additives, taking especially into account the priorities, procedures and deadlines that are enshrined in Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives.

1.1.3. Interpretation of Terms of Reference

The Panel on Food Additives and Nutrient Sources added to Food (ANS) described its risk assessment paradigm in the Guidance for submission for food additive evaluations in 2012 (EFSA ANS Panel, 2012). This Guidance states, that in carrying out its risk assessments, the Panel sought to define a health-based guidance value, e.g. an acceptable daily intake (ADI) (IPCS, 2004) applicable to the general population. According to the definition above, the ADI as established for the general population does not apply to infants below 12 weeks of age (JECFA, 1978; SCF, 1998). In this context, the re-evaluation of the use of starch sodium octenyl succinate (E 1450) in food for infants below 12 weeks represents a special case for which specific recommendations were given by the Joint FAO/WHO

¹ Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16–33.

² Commission Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives. OJ L 80, 26.3.2010, p. 19–27.

³ COM(2001) 542 final.

Expert Committee on Food Additives (JECFA, 1972a, 1978) and by the SCF (1996, 1998). The Panel endorsed these recommendations.

In the current EU legislation (Regulation (EC) No 1333/2008), use levels of additives in food for infants under the age of 12 weeks in categories 13.1.1 and 13.1.5.1⁴ (Annex II) and uses of food additives in nutrient preparations for use in food for infants under the age of 12 weeks and maximum levels for the carry-over from these uses (Annex III, Part 5, section B) are included. The Panel considers that these uses would require a specific risk assessment in line with the recommendations given by JECFA and the SCF and endorsed by the Panel in its current Guidance for submission for food additives evaluations (EFSA ANS Panel, 2012). Therefore, risk assessments for the general population are not considered applicable for infants under the age of 12 weeks and will be performed separately.

This re-evaluation refers exclusively to the uses of modified starches as food additives in food, including food supplements and does not include a safety assessment of other uses of modified starches.

1.2. Information on existing authorisations and evaluations

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) are listed in Commission Regulation (EC) No 1333/2008 as authorised food additives in the EU and have been previously evaluated by JECFA and by the SCF. An acceptable daily intake (ADI) 'not specified' was allocated by both committees in their evaluations.

A group of modified starches was evaluated by the SCF in 1976 (SCF, 1976). The starches E 1404–1422 were assigned to group B: 'starches may be used temporarily until 31 December 1980 but the numbers and amounts used should be limited in infant foods. For these latter foods every effort should be made to work within a maximum of 3.5%. If technologically necessary for the manufacture of certain products, the Committee could accept a maximum of 5%'. The starches E 1440 and E 1442 were assigned to Group C (starches that should not be allowed in infant foods). They were acceptable for use in food, other than that prepared for infants, on a temporary basis until 31 December 1980, subject to a limit for total chlorohydrins of 1 mg/kg in the relevant specifications.

The group of modified starches was evaluated a second time by the SCF in 1981 (SCF, 1982). Additional short-term, long-term and reproductive toxicity studies on starches previously classified into group B or C⁵ were reviewed. The SCF considered the appearance and mechanism of corticomedullary and of pelvic nephrocalcinosis (PN) as a finding to be specific for the rat as the most sensitive species and to have little relevance for the safety assessment of modified starches for man. The SCF considered that E 1440 and E 1442 could be transferred to group B provided residues of chlorohydrin did not exceed 0.1 mg/kg as determined by an agreed method. The Panel noted that according to the current specifications, the residues of propylene chlorohydrin should not exceed 1 mg/kg (Commission Regulation (EU) No 231/2012⁶).

Starch sodium octenyl succinate (E 1450; octenyl succinic anhydride (OSA)-modified starch) was evaluated by the SCF in 1990 (SCF, 1994), and acetylated oxidised starch (E 1451) was evaluated by the SCF in 1995 (SCF, 1997) and were included among the other modified starches in group B, for which use was considered acceptable and for which the establishment of individual ADIs was judged by the SCF to be unnecessary, provided the technological usage remained at present-day levels.

The group of 'modified starches' was discussed by JECFA in 1969 (JECFA, 1970), in 1971 (JECFA, 1972b), in 1973 (JECFA, 1974c) and in 1982 (JECFA, 1982a). The group 'modified starches' comprised the following substances: E 1404, E 1410, E 1412, E 1413, E 1414, E 1420, E 1422, E 1440, E 1442 and E 1450. At this meeting, JECFA established an ADI 'not specified' for all modified starches listed

⁴ Food category 13.1.1: Infant formulae as defined by Directive 2006/141/EC; Food category 13.1.5.1: Dietary foods for infants for special medical purposes and special formulae for infants. This interpretation also applies to those food additives in food category 13.1.5.2 (Dietary foods for babies and young children for special medical purposes as defined in Directive 1999/21/EC) for which exceptional uses in food for infants under the age of 12 weeks are indicated.

⁵ Group A: modified starches that may be used without special restrictions.

⁶ Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p. 1–295.

above except for acetylated oxidised starch (E 1451), for which an ADI 'not specified' was established at the 57th JECFA meeting (JECFA, 2002a).

Starch sodium octenyl succinate (OSA-modified starch; E 1450) was evaluated at the 79th JECFA meeting (JECFA, 2015). Since the 26th meeting, where an ADI 'not specified' was assigned to OSA-modified starch, new data became available, including a 90-day oral toxicity study, genotoxicity studies and a long-term toxicity and carcinogenicity study. The Committee confirmed the low toxicity of the additive and also confirmed the ADI 'not specified' for the general population. The Committee 'took into account the overall low toxicity of OSA-modified starch, the conservatism in the no-observed-adverse-effect level (NOAEL), which was the highest dose tested in a study in neonatal animals, and in the exposure assessments, as well as the supporting evidence from clinical trials and post-marketing surveillance and concluded that the consumption of OSA-modified starch in infant formula or formula for special medical purposes intended for infants is not of concern at use levels up to 20 g/L'.

Additionally, in 2010, the EFSA Panel on Dietetic Products, Nutrition and Allergies (EFSA NDA Panel, 2010) evaluated phosphated distarch phosphate for use as a novel food ingredient. The starch was prepared with a novel maize starch source. The NDA Panel concluded that the novel ingredient was safe at the proposed conditions of use and intake levels.

In 1979, the Federation of American Societies for Experimental Biology (FASEB, 1979) evaluated starch and modified starches for status as generally recognised as safe (GRAS) food ingredients.

In 2008, the safety of aluminium from dietary intake has been evaluated by the EFSA Panel on Food Additives, Flavourings, Processing Aids and Food Contact Materials (AFC). The Panel established a tolerable weekly intake (TWI) for aluminium of 1 mg/kg body weight (bw) per week (EFSA, 2008).

In 2011, JECFA established a provisional tolerable weekly intake (PTWI) for aluminium of 2 mg/kg bw per week (JECFA, 2012).

2. Data and methodologies

2.1. Data

The ANS Panel was not provided with a newly submitted dossier. EFSA launched public calls for data,^{7–10} to collect relevant information from interested parties.

The Panel based its assessment on information submitted to EFSA following the public calls for data, information from previous evaluations and additional available literature up to the date of the last Working Group (WG) meeting before the adoption of the opinion.¹¹ Attempts were made to retrieve relevant original study reports on which previous evaluations or reviews were based, however, not always these were available to the Panel.

The EFSA Comprehensive European Food Consumption Database (Comprehensive Database¹²) was used to estimate the dietary exposure.

The Mintel's Global New Products Database (GNPD) is an online database which was used for checking the labelling of products containing modified starches within the EU's food products, as GNPD shows the compulsory ingredient information presented in the labelling of products.

2.2. Methodologies

The assessment was conducted in line with the principles described in the EFSA Guidance on transparency in the scientific aspects of risk assessment (EFSA Scientific Committee, 2009) and following the relevant existing Guidances from the EFSA Scientific Committee.

The ANS Panel assessed the safety of modified starches (E 1404, E 1410, E 1412, E 1413, E 1414, E 1420, E 1422, E 1440, E 1442, E 1450, E 1451 and E 1452) as food additives in line with the principles laid down in Regulation (EU) 257/2010 and the relevant guidance documents: Guidance on

⁷ Call for scientific data on miscellaneous food additives permitted in the EU and belonging to several functional classes. Published: 9 June 2010. Available online: <http://www.efsa.europa.eu/it/dataclosed/call/ans100609>

⁸ Call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Published: 12 October 2015. Available online: <https://www.efsa.europa.eu/it/data/call/151012>

⁹ Call for technical data on certain starches and celluloses authorised as food additives in the EU. Published: 11 February 2016. Available online: <https://www.efsa.europa.eu/it/data/call/160211>

¹⁰ Call for usage level data on starch aluminium octenyl succinate (E 1452) in food intended for human consumption. Published: 20 October 2016. Available online: <https://www.efsa.europa.eu/en/data/call/161020>

¹¹ 2 May 2017.

¹² Available online: <http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm>

submission for food additive evaluations by the SCF (2001) and taking into consideration the Guidance for submission for food additive evaluations in 2012 (EFSA ANS Panel, 2012).

When the test substance was administered in the feed or in the drinking water, but doses were not explicitly reported by the authors as mg/kg bw per day based on actual feed or water consumption, the daily intake was calculated by the Panel using the relevant default values as indicated in the EFSA Scientific Committee Guidance document (EFSA Scientific Committee, 2012) for studies in rodents or, in the case of other animal species, by JECFA (2000). When in human studies in adults (aged above 18 years) the dose of the test substance administered was reported in mg/person per day, the dose in mg/kg bw per day was calculated by the Panel using a body weight of 70 kg as default for the adult population, as described in the EFSA Scientific Committee Guidance document (EFSA Scientific Committee, 2012).

Dietary exposure to modified starches from their use as food additives was estimated combining the food consumption data available within the EFSA Comprehensive European Food Consumption Database with the maximum permitted levels (MPLs) and/or reported use levels submitted to EFSA following a call for data. Different scenarios were used to calculate the exposure (see Section 3.7.1). Uncertainties in the exposure assessment were identified and discussed.

In the context of this re-evaluation, the Panel followed the conceptual framework for the risk assessment of certain food additives re-evaluated under Commission Regulation (EC) No 257/2010 (EFSA ANS Panel, 2014).

3. Assessment

3.1. Technical data

3.1.1. Identity of the substances

According to Regulation (EC) No 1333/2008, 'modified starches' are substances obtained by one or more chemical treatments of edible starches, which may have undergone a physical or enzymatic treatment, and may be acid or alkali thinned or bleached. According to the same regulation, the following are not considered to be food additives: white or yellow dextrin, roasted or dextrinated starch, starch modified by acid or alkali treatment, bleached starch, physically modified starch and starch treated by amylolytic enzymes. In contrary, JECFA considers dextrin roasted starch (INS No 1400), acid-treated starch (INS No 1401), alkaline-treated starch (INS No 1402), bleached starch (INS No 1403) and enzyme-treated starch (INS No 1405) as food additives, with separate specifications.

Starch typically consists of two polymers of glucose, namely amylose, with almost linear structure, and amylopectin, which is highly branched (EFSA NDA Panel, 2010). In amylose, the glucose monomers (pyranosic form) are linked by α -1,4-glycosidic links, while amylopectin contains additionally α -1,6-glycosidic bonds (Heyns, 1983). The chemical structure of both main components of starch is shown in Figure 1. Commercial starches are composed of about 20–25% amylose and 75–80% amylopectin (Heyns, 1983). High amylose starches typically consist of 50–80% amylose and 20–50% amylopectin.

Starch is deposited as insoluble microsize semicrystalline granules primarily in plants' storage tissues. The physicochemical properties of a given starch, including the dimensions of starch polymers and granules, can vary remarkably depending upon the plant source – even the same plant cultivar grown under different conditions – and possibly upon the starch extraction method (Jackson, 2003; Ratnayake and Jackson, 2003; BeMiller, 2004; Copeland et al., 2009). Therefore, polymer molecular weights (and the correlated degrees of polymerisation) can only indicatively be given (rounding-off to one figure): for amylopectins, molecular weight estimates have been reported to vary from 50×10^6 Da to 500×10^6 Da, with an average near 100×10^6 Da (higher values have been reported by Yoo and Jane, 2002); for amyloses, estimates appear to fall between 2×10^3 Da and $4,000 \times 10^3$ Da. Molecular weights of 50×10^6 Da and 200×10^3 Da for amylopectin and amylose, respectively, were reported (EFSA NDA Panel, 2010). In different varieties of rice, Ma et al. (2007) determined the weight-average molecular weight (M_w) of amylopectins to be between 40×10^6 and 300×10^6 , while Zhong et al. (2006) reported M_w estimates for starch with varying amylose content in the range from 60×10^6 to 130×10^6 . Due to starch chemical complexity, combinations of analytical techniques have been used to investigate the molecular organisation within starch granules (Zhong et al., 2006; Copeland et al., 2009): the type of analysis carried out can be responsible for

some of the differences in the reported molecular weights of amylopectins and amyloses. The limitations of the different methods for the determination of starch molecular weights have recently been reviewed by Harding et al. (2016).

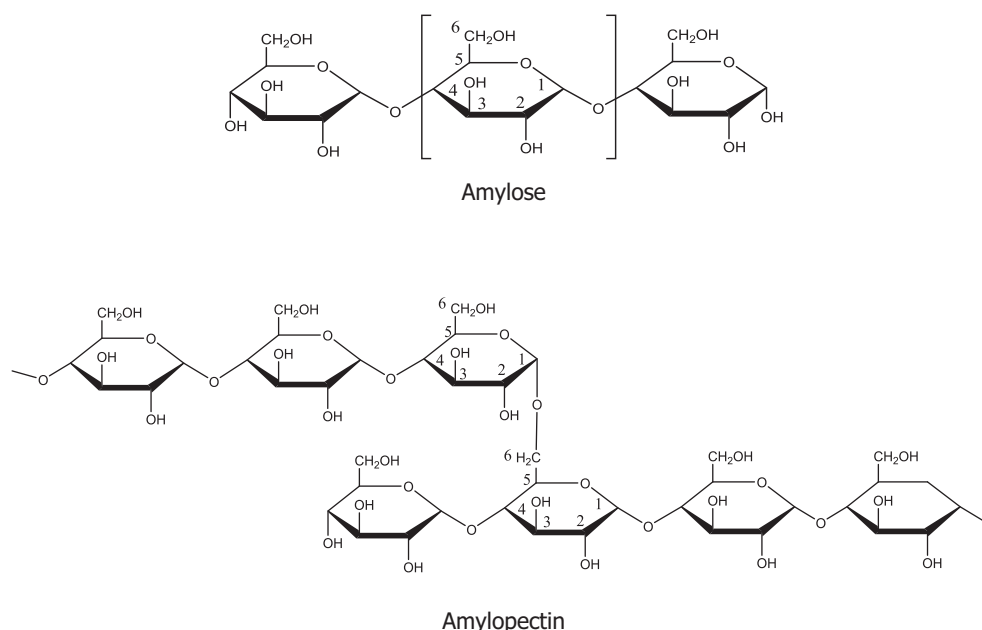


Figure 1: Structural formula of amylose and an example of branching in amylopectin

Starches for commercial use are generally produced from potatoes, cereals, or other sources (Documentation provided to EFSA n. 1). The chemical structure of starch provides a great possibility of modifications (Fortuna, 1991). The most common chemical modification of the so-called 'native' starches includes oxidation, esterification and etherification (Xie et al., 2005).

The identity 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) are summarised in Table 1.

Table 1: Identity of modified starches

Food additive	CAS No	EC no ^(a)	Synonyms
Oxidised starch (E 1404)	910452-67-4 ^(b)	–	Modified starch, INS No 1404; starch, oxidised
Monostarch phosphate (E 1410)	63100-01-6	–	Modified starch, INS No 1410; starch, dihydrogen phosphate
Distarch phosphate (E 1412)	55963-33-2	611-338-9	Modified starch, INS No 1412; starch, hydrogen phosphate
Phosphated distarch phosphate (E 1413)	11120-02-8	601-054-3	Modified starch, INS No 1413; starch, phosphate
Acetylated distarch phosphate (E 1414)	68130-14-3	–	Modified starch, INS No 1414; starch, hydrogen phosphate acetate
Acetylated starch (E 1420)	9045-28-7	618-556-3	Modified starch, INS No 1420; starch, acetate
Acetylated distarch adipate (E 1422)	63798-35-6	613-382-4	Modified starch, INS No 1422; starch, acetate hexanedioate
Hydroxypropyl starch (E 1440)	9049-76-7	618-565-2	Modified starch, INS No 1440; starch, 2-hydroxypropyl ether

Food additive	CAS No	EC no ^(a)	Synonyms
Hydroxypropyl distarch phosphate (E 1442)	53124-00-8	610-966-0	Modified starch, INS No 1442; starch, hydrogen phosphate, 2-hydroxypropyl ether
Starch sodium octenyl succinate (E 1450)	66829-29-6	–	Modified starch, INS No 1450; starch, hydrogen 2-(octen-1-yl)butanedioate, sodium salt; SSOS; OSA-modified starch
Acetylated oxidised starch (E 1451)	68187-08-6	614-359-1	Modified starch, INS No 1451; starch, acetylated oxidised
Starch aluminium octenyl succinate (E 1452)	9087-61-0	618-671-9	Starch, hydrogen 2-(octen-1-yl)butanedioate, aluminium salt; starch octenyl succinate aluminium salt

CAS: Chemical Abstract Service; EC: Enzyme Commission.

(a): According to the ECHA database (<https://echa.europa.eu/information-on-chemicals>), EC numbers with format 6xx-xxx-x have no official status and no legal significance.

(b): The Panel is aware that the CAS number 65996-62-5 is registered for a broadly defined oxidised starch: this CAS number appears to be paired with EC number 613-862-3.

The CAS Registry numbers and EC numbers reported in Table 1 were subject to confirmatory steps to minimise the uncertainty of an equivocal identification met in few cases. According to Starch Europe (Documentation provided to EFSA n. 2), 'it is not possible to easily match a CAS number directly to an E-number for modified starches', as the identity requirements for the E-numbering of food additives and for CAS registration of chemicals are different. The CAS registration scheme is not directly concerned with toxicological and health safety issues and can unintentionally allow that multiple, possibly erratic, or redundant entries exist for identification of the same or similar starch substances. In contrast, the primary aim of E-numbering is to reflect the food safety aspects of modified starches. As a consequence, Starch Europe members 'do not believe that CAS numbers are an appropriate parameter for defining or identifying a particular modified starch. Furthermore, any CAS number reference to an E-number may suggest unrealistic and misleading safety aspects for the respective CAS number. Therefore, it is not anticipated that there would be any added value in assigning a particular CAS number reference to an E-number identity and it would not be appropriate to restrict or confine a modified starch with only certain CAS numbers'.

Although Starch Europe's observations reflect possible difficulties in attributing unequivocal CAS identification numbers to complex chemical structures such as modified starches, yet, the Panel noted that attributing CAS numbers, which are as reliable as possible, to such structures is probably the 'best' available way for a reasonably accurate identification of the chemicals.

According to Commission Regulation (EU) No 231/2012, all modified starches described in the present document are white or nearly white powder or granules or (if pregelatinised) flakes, amorphous powder or coarse particles. The particle size of commercial starches has been reported to be greater than 0.5 µm (Documentation provided to EFSA n. 2).

3.1.1.1. Oxidised starch (E 1404)

According to Commission Regulation (EU) No 231/2012, oxidised starch is starch treated with sodium hypochlorite. Oxidised starches are normally whiter than unmodified starches, because pigments as minor residues in the molecules are bleached (Xie et al., 2005). When heated in water, oxidised starches form clear fluid solutions. On cooling, however, the solutions are more stable or resistant to thickening and forming gels or pastes than their acid-converted counterparts (Wurzburg, 2006).

3.1.1.2. Monostarch phosphate (E 1410)

According to Commission Regulation (EU) No 231/2012, monostarch phosphate is starch esterified with orthophosphoric acid, or sodium or potassium orthophosphate or sodium tripolyphosphate. Based on structural evaluations, the phosphoric ester groups are mainly attached to C-6 and to a lesser extent to C-2 and C-3 of the glucopyranose units (JECFA, 1974a; Lim and Seib, 1993). The content of residual phosphate is limited by the Commission Regulation (EU) to 0.5% bound P for wheat and potato starches, and 0.4% for starches from other sources.

Compared to native starch, the starch phosphates display greater water solubility and water-binding capacity, and both characteristics increase with increasing phosphate substitution (Fortuna et al., 1990). Phosphorylation causes higher viscosities and greater clarity of the dispersions (Wurzburg, 2006). Higher degrees of phosphate lower the pasting temperature, while maximum viscosity is slightly increased (Fortuna et al., 1990).

3.1.1.3. Distarch phosphate (E 1412)

In Commission Regulation (EU) No 231/2012, distarch phosphate is defined as starch cross-linked with sodium trimetaphosphate or phosphorus oxychloride. Thereby, phosphate groups form crosswise bindings between the neighbouring chains of glucose rests (Fortuna et al., 1992). The approximate rate of phosphate groups per glucopyranose unit is, depending on the production process, 1:620 or 1:100 (JECFA, 1974b). The content of residual phosphate is limited by the Commission Regulation (EU) No 231/2012 to 0.5% bound P for wheat and potato starches, and 0.4% for starches from other sources.

An example of a phosphate cross-link between two glucopyranose units in distarch phosphate is shown in Figure 2.

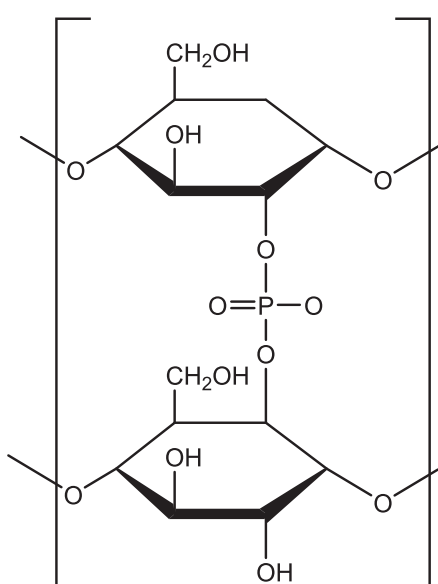


Figure 2: Structural representation of a phosphate cross-link between two glucopyranose units in distarch phosphate

In aqueous solutions, starch with a low level of cross-linking shows higher viscosity than native starch. Increase of the cross-linking level decreases the peak viscosity (Lewandowicz et al., 2004; Xie et al., 2005). The effects of acidification are depending on the level of cross-linking: while in slightly cross-linked starch preparations viscosity decreases, in medium and high cross-linked preparations viscosity is higher at pH 3.5 than at pH 5.5 (Lewandowicz et al., 2004). The properties are depending on the origin of the starch, both water-binding capacity and viscosity of the gels being higher in cereal starches than in potato starch (Fortuna, 1991).

3.1.1.4. Phosphated distarch phosphate (E 1413)

According to Commission Regulation (EU) No 231/2012, phosphated distarch phosphate is starch having undergone a combination of treatments as described for monostarch phosphate and for distarch phosphate. The content of residual phosphate is limited by the Commission Regulation (EU) No 231/2012 to 0.5% bound P for wheat and potato starches, and 0.4% for starches from other sources. Phosphorus in the phosphate cross-links and in the esterified groups represents 0.01% and 0.32%, respectively (EFSA NDA Panel, 2010).

Phosphated distarch phosphate (E 1413) consists of covalently linked phosphated starch ($\geq 70\%$), residual unreacted starch (7–14%), water (10–14%), lipids (0.8%) and proteins (0.8%) (EFSA NDA Panel, 2010).

3.1.1.5. Acetylated distarch phosphate (E 1414)

In Commission Regulation (EU) No 231/2012, acetylated distarch phosphate is defined as starch cross-linked with sodium trimetaphosphate or phosphorus oxychloride and esterified by acetic anhydride or vinyl acetate. According to the EU purity criteria, the content of residual phosphate is limited to 0.14% bound P for wheat and potato starches, 0.04% for starches from other sources, and to 2.5% acetyl groups.

The behaviour of acetylated distarch phosphate in aqueous solutions is similar to that of distarch phosphate (E 1412). Viscosity decreases with increasing level of cross-linking. The effects of acidification are depending on the level of cross-linking: while in slightly cross-linked starch preparations viscosity decreases, in medium and high cross-linked preparations viscosity is higher at pH 3.5 than at pH 5.5 (Lewandowicz et al., 2004).

3.1.1.6. Acetylated starch (E 1420)

In Commission Regulation (EU) No 231/2012, acetylated starch is defined as starch esterified with acetic anhydride or vinyl acetate. According to the EU purity criteria, the content of acetyl groups is limited to a maximum of 2.5%, corresponding to a maximum degree of substitution (DS) of 0.1. The level of acetyl groups is significantly lower in most commercial products (JECFA, 1982f; Wurzburg, 2006).

Compared to native starch, acetylation increases the water solubility and water-binding capacity (Khalil et al., 1995; Bello-Perez et al., 2000; Berski et al., 2011). The influence of acetylation on viscosity of pastes is inconsistent: while in some cases lower viscosity was observed, some authors observed increased viscosity in the case of acetyl starches (Berski et al., 2011). Acetylated starch is stable against hydrolysis under mild and moderate acidic conditions. However, it is sensitive to alkaline hydrolysis: at pH 11 complete deacetylation is achieved after 4 h at 25°C (Wurzburg, 2006).

3.1.1.7. Acetylated distarch adipate (E 1422)

According to Commission Regulation (EU) No 231/2012, acetylated distarch adipate is starch cross-linked with adipic anhydride and esterified with acetic anhydride. Thereby, adipate groups form crosswise bindings between the adjacent chains of glucose rests. According to the EU purity criteria, the content of adipate is limited to a maximum of 0.135%. In general, the concentration is below 0.09%, corresponding to one adipyl molecule per 1,000 glucose units (JECFA, 1982b; Wurzburg, 2006). The content of acetyl groups is limited by the regulation to 2.5%.

An exemplified structure of distarch adipate is shown in Figure 3.

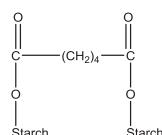


Figure 3: Structural representation of distarch adipate; the acetyl groups present in acetylated distarch adipate are not shown (Reproduced with permission of CCR Press, from Xie et al., 2005)

In water, acetylated distarch adipate forms viscous solutions. Viscosity decreases slowly with pH decrease. Investigations with potato starch revealed improved acid resistance, salt tolerance and good viscosity breakdown properties of acetylated distarch adipate, compared to the native starch (Luo et al., 2009).

3.1.1.8. Hydroxypropyl starch (E 1440)

In Commission Regulation (EU) No 231/2012, hydroxypropyl starch is defined as starch etherified with propylene oxide. The DS is a maximum of four ether linkages per 10 glucopyranose units (i.e. 13.3%) if 25% propylene oxide is used, and four to six ether linkages per 100 glucopyranose units if 5% propylene oxide is used (JECFA, 1982d). According to Xie et al. (2005), commercial food grade starches have hydroxypropyl levels in the range of 3.3–11.5%. Under the alkaline conditions utilised, propylene oxide reacts with starch with a nucleophilic bimolecular substitution mechanism (S_N2 -type reaction): hydroxypropylation takes place primarily on the secondary hydroxyl at the C-2 position (Xie et al., 2005; Wurzburg, 2006). The hydroxypropyl groups have been reported to be distributed with a

ratio of 7:2:1 at, respectively, the C-2, C-3 and C-6 positions of the glucose units (the aforesaid positions are also identified as 2-O, 3-O, and 6-O) (Xie et al., 2005).

An exemplified structure of hydroxypropyl starch is shown in Figure 4.

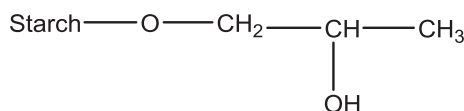


Figure 4: Structural representation of hydroxypropyl starch (Reproduced with permission of CCR Press, from Xie et al., 2005)

In aqueous media, hydroxypropyl starch forms viscous solutions, viscosity being highly dependent on the shear rate (Vorwergh et al., 2004). The pasting temperature decreases with an increase of the level of hydroxypropyl substitution (Xie et al., 2005). At a certain level of substitution, the starches become cold water-swelling (Xie et al., 2005).

3.1.1.9. Hydroxypropyl distarch phosphate (E 1442)

According to Commission Regulation (EU) No 231/2012, hydroxypropyl distarch phosphate is starch cross-linked with sodium trimetaphosphate or phosphorus oxychloride and etherified with propylene oxide. Thereby, phosphate groups form crosswise bindings between the neighbouring chains of glucose rests. According to the EU purity criteria, the content of phosphorus is limited to 0.04% and 0.14% bound P (depending on the origin of the starch), and to 7% hydroxypropyl groups.

3.1.1.10. Starch sodium octenyl succinate (E 1450)

In Commission Regulation (EU) No 231/2012, starch sodium octenyl succinate is defined as starch esterified with octenylsuccinic anhydride. According to the EU purity criteria, the content of octenylsuccinyl groups is limited to a maximum of 3% and the content of octenylsuccinic acid residue to a maximum of 0.3%. According to JECFA, the product has a DS of 0.02 (JECFA, 1982g).

An exemplified structure of starch sodium octenyl succinate is shown in Figure 5.

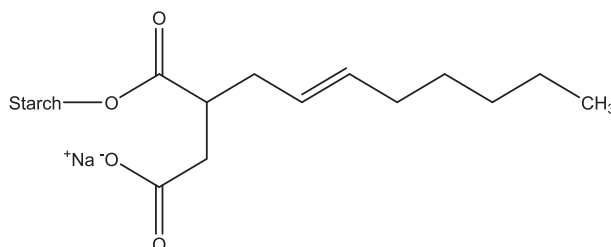


Figure 5: Structural representation of starch sodium octenyl succinate (Documentation provided to EFSA n. 1)

The free carboxylate group in the starch sodium octenyl succinate molecule increases the water-holding power, the tendency to swell in cold water and the viscosity. The capability of cold water-swelling increases when the level of substitution increases. Viscosity is highest at neutral pH and is considerably reduced in acid media (Xie et al., 2005).

3.1.1.11. Acetylated oxidised starch (E 1451)

According to Commission Regulation (EU) No 231/2012, acetylated oxidised starch is starch treated with sodium hypochlorite followed by esterification with acetic anhydride.

The water solubility of acetylated oxidised starch increases with increasing acetyl content (Khalil et al., 1995). Acetylation of oxidised starch enhances gel strength and clarity, resulting in a gummy, clear jelly. The substance hydrolyses slowly in the presence of strong acids (JECFA, 2002b).

3.1.1.12. Starch aluminium octenyl succinate (E 1452)

In Commission Regulation (EU) No 231/2012, starch aluminium octenyl succinate is defined as starch esterified with octenylsuccinic anhydride and treated with aluminium sulfate. According to the EU purity criteria, the content of octenylsuccinyl groups is limited to a maximum of 3%, octenylsuccinic acid residue to a maximum of 0.3% and aluminium to a maximum of 0.3%. The chemical structure is

equal to that of starch sodium octenyl succinate (E 1450), except that aluminium is present as cation (Nair and Yamarik, 2002).

Compared to native starch, starch aluminium octenyl succinate has a reduced tendency to associate in solution, lose clarity and form gels. Due to the introduction of the succinate ester groups, the substance has polyelectrolyte properties (Nair and Yamarik, 2002).

3.2. Specifications

Specifications for modified starches (E 1404, E 1410, E 1412, E 1413, E 1414, E 1420, E 1422, E 1440, E 1442, E 1450, E 1451 and E 1452) as defined in Commission Regulation (EU) No 231/2012 and by JECFA (2016a) are listed in Tables 2–13.

3.2.1. Oxidised starch (E 1404)

Table 2: Specifications for oxidised starch (E 1404) according to Commission Regulation (EU) No 231/2012 and JECFA (2016a)

	Commission Regulation (EU) No 231/2012	JECFA (2016a)
Definition	Oxidised starch is starch treated with sodium hypochlorite	Oxidised starch is a modified starch. It is obtained by treatment of food starch in accordance with good manufacturing practice with sodium hypochlorite. Oxidation involves the deliberate production of carboxyl groups Oxidised starch may additionally be subjected to acid, alkali, enzyme or bleaching treatment in accordance with good manufacturing practice
Description	White or nearly white powder or granules or (if pregelatinised) flakes, amorphous powder or coarse particles	White or nearly white powder or granules or (if pregelatinised) flakes, or amorphous powder or coarse particles
Identification		
Microscopic observation	Passes test (if not pregelatinised)	Passes test
Iodine staining	Passes test (dark blue to light red colour)	Passes test
Solubility	–	Insoluble in cold water (if not pregelatinised); forming typical colloidal solutions with viscous properties in hot water; insoluble in ethanol
Copper reduction	–	Passes test
Test for hypochlorite oxidised starch	–	Passes test
Purity		
Loss on drying	Not more than 15.0% for cereal starch Not more than 21.0% for potato starch Not more than 18.0% for other starches	Cereal starch: not more than 15.0% Potato starch: not more than 21.0% Other starches: not more than 18.0% (120°, 4 h, vacuum not exceeding 100 mmHg)
Carboxyl groups	Not more than 1.1% (on an anhydrous basis)	Not more than 1.3% on the dried basis
Sulfur dioxide	Not more than 50 mg/kg for modified cereal starches (on an anhydrous basis) Not more than 10 mg/kg for other modified starches, unless otherwise specified (on an anhydrous basis)	Not more than 50 mg/kg on the dried basis for modified cereal starches Not more than 10 mg/kg on the dried basis for other modified starches
Arsenic	Not more than 1 mg/kg	–
Lead	Not more than 2 mg/kg (on an anhydrous basis)	Not more than 2 mg/kg on the dried basis
Mercury	Not more than 0.1 mg/kg	–
Manganese	–	Not more than 50 mg/kg on the dried basis

3.2.2. Monostarch phosphate (E 1410)

Table 3: Specifications for monostarch phosphate (E 1410) according to Commission Regulation (EU) No 231/2012 and JECFA (2016a)

	Commission Regulation (EU) No 231/2012	JECFA (2016a, tentative specifications)
Definition	Monostarch phosphate is starch esterified with orthophosphoric acid, or sodium or potassium orthophosphate or sodium tripolyphosphate	Monostarch phosphate is a modified starch. It is obtained by esterification of food starch with orthophosphoric acid, or sodium or potassium orthophosphate, or sodium tripolyphosphate in accordance with good manufacturing practice. This treatment results in partial substitution in the 2, 3- or 6- position of the anhydroglucose unit unless the 6-position is occupied for branching. Monostarch phosphate may additionally be subjected to acid, alkali, enzyme or bleaching treatment in accordance with good manufacturing practice
Description	White or nearly white powder or granules or (if pregelatinised) flakes, amorphous powder or coarse particles	White or nearly white powder or granules or (if pregelatinised) flakes, or amorphous powder or coarse particles
Identification		
Solubility	—	Insoluble in cold water (if not pregelatinised); forming typical colloidal solutions with viscous properties in hot water; insoluble in ethanol
Microscopic observation	Passes test (if not pregelatinised)	Passes test
Iodine staining	Passes test (dark blue to light red colour)	Passes test
Copper reduction	—	Passes test
Purity		
Loss on drying	Not more than 15.0% for cereal starch Not more than 21.0% for potato starch Not more than 18.0% for other starches	Cereal starch: not more than 15.0% Potato starch: not more than 21.0% Other starches: not more than 18.0% (120°, 4 h, vacuum not exceeding 100 mmHg)
Residual phosphate	Not more than 0.5% (as P) for wheat or potato starch (on an anhydrous basis) Not more than 0.4% (as P) for other starches (on an anhydrous basis)	Not more than 0.5% on the dried basis for potato or wheat starches Not more than 0.4% on the dried basis for other starches
Carboxyl groups	—	Not more than 0.1% on the dried basis
Sulfur dioxide	Not more than 50 mg/kg for modified cereal starches (on an anhydrous basis) Not more than 10 mg/kg for other modified starches, unless otherwise specified (on an anhydrous basis)	Not more than 50 mg/kg on the dried basis for modified cereal starches Not more than 10 mg/kg on the dried basis for other modified starches
Arsenic	Not more than 1 mg/kg	—
Lead	Not more than 2 mg/kg (on an anhydrous basis)	Not more than 2 mg/kg on the dried basis
Mercury	Not more than 0.1 mg/kg	—
Manganese	—	Not more than 50 mg/kg on the dried basis

3.2.3. Distarch phosphate (E 1412)

Table 4: Specifications for distarch phosphate (E 1412) according to Commission Regulation (EU) No 231/2012 and JECFA (2016a)

	Commission Regulation (EU) No 231/2012	JECFA (2016a)
Definition	Distarch phosphate is starch cross-linked with sodium trimetaphosphate or phosphorus oxychloride	Distarch phosphate is a modified starch. It is obtained by esterification of food starch with sodium trimetaphosphate or phosphorus oxychloride in accordance with good manufacturing practice. This treatment results in cross-linking, where a polyfunctional substituting agent, such as phosphorus oxychloride, connects two chains. The structure can be represented by: Starch–O–R–O–Starch, where R = cross-linking group and Starch refers to the linear and/or branched structure. Distarch phosphate may additionally be subjected to acid, alkali, enzyme or bleaching treatment in accordance with good manufacturing practice
Description	White or nearly white powder or granules or (if pregelatinised) flakes, amorphous powder or coarse particles	White or nearly white powder or granules or (if pregelatinised) flakes, or amorphous powder or coarse particles
Identification		
Solubility	–	Insoluble in cold water (if not pregelatinised); forming typical colloidal solutions with viscous properties in hot water; insoluble in ethanol
Microscopic observation	Passes test (if not pregelatinised)	Passes test
Iodine staining	Passes test (dark blue to light red colour)	Passes test
Copper reduction	–	Passes test
Purity		
Loss on drying	Not more than 15.0% for cereal starch Not more than 21.0% for potato starch Not more than 18.0% for other starches	Cereal starch: not more than 15.0% Potato starch: not more than 21.0% Other starches: not more than 18.0% (120°, 4 h, vacuum not exceeding 100 mmHg)
Residual phosphate	Not more than 0.5% (as P) for wheat or potato starch (on an anhydrous basis) Not more than 0.4% (as P) for other starches (on an anhydrous basis)	Not more than 0.5% on the dried basis for potato or wheat starch Not more than 0.4% on the dried basis for other starches
Carboxyl groups	–	Not more than 0.1% on the dried basis
Sulfur dioxide	Not more than 50 mg/kg for modified cereal starches (on an anhydrous basis) Not more than 10 mg/kg for other modified starches, unless otherwise specified (on an anhydrous basis)	Not more than 50 mg/kg on the dried basis for modified cereal starches Not more than 10 mg/kg on the dried basis for other modified starches
Arsenic	Not more than 1 mg/kg	–
Lead	Not more than 2 mg/kg (on an anhydrous basis)	Not more than 2 mg/kg on the dried basis
Mercury	Not more than 0.1 mg/kg	–
Manganese	–	Not more than 50 mg/kg on the dried basis

3.2.4. Phosphated distarch phosphate (E 1413)

Table 5: Specifications for phosphated distarch phosphate (E 1413) according to Commission Regulation (EU) No 231/2012 and JECFA (2016a)

	Commission Regulation (EU) No 231/2012	JECFA (2016a)
Definition	Phosphated distarch phosphate is starch having undergone a combination of treatments as described for monostarch phosphate and for distarch phosphate	Phosphated distarch phosphate is a modified starch. It is obtained by esterification/cross-linking of food starch with sodium trimetaphosphate or phosphorus oxychloride combined with esterification with orthophosphoric acid, or sodium or potassium orthophosphate, or sodium tripolyphosphate, in accordance with good manufacturing practice. The esterification results in partial substitution in the 2, 3- or 6- position of the anhydroglucose unit unless the 6-position is occupied for branching. In the case of cross-linking, where a polyfunctional substituting agent, such as phosphorus oxychloride, connects two chains, the structure can be represented by: Starch-O-R-O-Starch, where R = cross-linking group and Starch refers to the linear and/or branched structure. Phosphated distarch phosphate may additionally be subjected to acid, alkali, enzyme or bleaching treatment in accordance with good manufacturing practice
Description	White or nearly white powder or granules or (if pregelatinised) flakes, amorphous powder or coarse particles	White or nearly white powder or granules or (if pregelatinised) flakes, or amorphous powder or coarse particles
Identification		
Solubility	–	Insoluble in cold water (if not pregelatinised); forming typical colloidal solutions with viscous properties in hot water; insoluble in ethanol
Microscopic observation	Passes test (if not pregelatinised)	Passes test
Iodine staining	Passes test (dark blue to light red colour)	Passes test
Copper reduction	–	Passes test
Purity		
Loss on drying	Not more than 15.0% for cereal starch Not more than 21.0% for potato starch Not more than 18.0% for other starches	Cereal starch: not more than 15.0% Potato starch: not more than 21.0% Other starches: not more than 18.0% (120°, 4 h, vacuum not exceeding 100 mmHg)
Residual phosphate	Not more than 0.5% (as P) for wheat or potato starch (on an anhydrous basis) Not more than 0.4% (as P) for other starches (on an anhydrous basis)	Not more than 0.5% on the dried basis for potato or wheat starch Not more than 0.4% on the dried basis for other starches
Carboxyl groups	–	Not more than 0.1% on the dried basis
Sulfur dioxide	Not more than 50 mg/kg for modified cereal starches (on an anhydrous basis) Not more than 10 mg/kg for other modified starches, unless otherwise specified (on an anhydrous basis)	Not more than 50 mg/kg on the dried basis for modified cereal starches Not more than 10 mg/kg on the dried basis for other modified starches
Arsenic	Not more than 1 mg/kg	–
Lead	Not more than 2 mg/kg (on an anhydrous basis)	Not more than 2 mg/kg on the dried basis
Mercury	Not more than 0.1 mg/kg	–
Manganese	–	Not more than 50 mg/kg on the dried basis

3.2.5. Acetylated distarch phosphate (E 1414)

Table 6: Specifications for acetylated distarch phosphate (E 1414) according to Commission Regulation (EU) No 231/2012 and JECFA (2016a)

	Commission Regulation (EU) No 231/2012	JECFA (2016a)
Definition	Acetylated distarch phosphate is starch cross-linked with sodium trimetaphosphate or phosphorus oxychloride and esterified by acetic anhydride or vinyl acetate	Acetylated distarch phosphate is a modified starch. It is obtained by esterification/cross-linking of food starch with sodium trimetaphosphate or phosphorus oxychloride combined with esterification with acetic anhydride or vinyl acetate in accordance with good manufacturing practice. Acetylation results in substitution of hydroxyl groups with acetyl esters. In cases of cross-linking, where a polyfunctional substituting agent, such as phosphorus oxychloride, connects two chains, the structure can be represented by: Starch–O–R–O–Starch, where R = cross-linking group and Starch refers to the linear and/or branched structure. Acetylated distarch phosphate may additionally be subjected to acid, alkali, enzyme or bleaching treatment in accordance with good manufacturing practice
Description	White or nearly white powder or granules or (if pregelatinised) flakes, amorphous powder or coarse particles	White or nearly white powder or granules or (if pregelatinised) flakes, or amorphous powder or coarse particles
Identification		
Microscopic observation	Passes test (if not pregelatinised)	Passes test
Iodine staining	Passes test (dark blue to light red colour)	Passes test
Solubility	–	Insoluble in cold water (if not pregelatinised); forming typical colloidal solutions with viscous properties in hot water; insoluble in ethanol
Copper reduction	–	Passes test
Specific reaction for acetyl groups	–	Passes test
Ester groups	–	Passes test
Purity		
Loss on drying	Not more than 15.0% for cereal starch Not more than 21.0% for potato starch Not more than 18.0% for other starches	Cereal starch: not more than 15.0% Potato starch: not more than 21.0% Other starches: not more than 18.0% (120°, 4 h, vacuum not exceeding 100 mmHg)
Acetyl groups	Not more than 2.5% (on an anhydrous basis)	Not more than 2.5% on the dried basis
Carboxyl groups	–	Not more than 0.1% on the dried basis
Residual phosphate	Not more than 0.14% (as P) for wheat or potato starch (on an anhydrous basis) Not more than 0.04% (as P) for other starches (on an anhydrous basis)	Not more than 0.14% on the dried basis for potato and wheat starch Not more than 0.04% on the dried basis for other starches
Vinyl acetate	Not more than 0.1 mg/kg (on an anhydrous basis)	Not more than 0.1 mg/kg

	Commission Regulation (EU) No 231/2012	JECFA (2016a)
Sulfur dioxide	Not more than 50 mg/kg for modified cereal starches (on an anhydrous basis) Not more than 10 mg/kg for other modified starches, unless otherwise specified (on an anhydrous basis)	Not more than 50 mg/kg on the dried basis for modified cereal starches Not more than 10 mg/kg on the dried basis for other modified starches
Arsenic	Not more than 1 mg/kg	–
Lead	Not more than 2 mg/kg (on an anhydrous basis)	Not more than 2 mg/kg on the dried basis
Mercury	Not more than 0.1 mg/kg	–
Manganese	–	Not more than 50 mg/kg on the dried basis

3.2.6. Acetylated starch (E 1420)

Table 7: Specifications for acetylated starch (E 1420) according to Commission Regulation (EU) No 231/2012 and JECFA (2016a)

	Commission Regulation (EU) No 231/2012	JECFA (2016a)
Definition	Acetylated starch is starch esterified with acetic anhydride or vinyl acetate	Starch acetate is a modified starch. It is obtained by esterification of food starches with acetic anhydride or vinyl acetate in accordance with good manufacturing practice. The esterification/acetylation results in substitution of hydroxyl groups with acetyl esters. Starch acetate may additionally be subjected to acid, alkali, enzyme or bleaching treatment in accordance with good manufacturing practice
Description	White or nearly white powder or granules or (if pregelatinised) flakes, amorphous powder or coarse particles	White or nearly white powder or granules or (if pregelatinised) flakes, or amorphous powder or coarse particles
Identification		
Microscopic observation	Passes test (if not pregelatinised)	Passes test
Iodine staining	Passes test (dark blue to light red colour)	Passes test
Solubility	–	Insoluble in cold water (if not pregelatinised); forming typical colloidal solutions with viscous properties in hot water; insoluble in ethanol
Copper reduction	–	Passes test
Specific reaction for acetyl groups	–	Passes test
Ester groups	–	Passes test
Purity		
Loss on drying	Not more than 15.0% for cereal starch Not more than 21.0% for potato starch Not more than 18.0% for other starches	Cereal starch: not more than 15.0% Potato starch: not more than 21.0% Other starches: not more than 18.0% (120°, 4 h, vacuum not exceeding 100 mmHg)
Acetyl groups	Not more than 2.5% (on an anhydrous basis)	Not more than 2.5% on the dried basis
Carboxyl groups	–	Not more than 0.1% on the dried basis
Vinyl acetate	Not more than 0.1 mg/kg (on an anhydrous basis)	Not more than 0.1 mg/kg

	Commission Regulation (EU) No 231/2012	JECFA (2016a)
Sulfur dioxide	Not more than 50 mg/kg for modified cereal starches (on an anhydrous basis) Not more than 10 mg/kg for other modified starches, unless otherwise specified (on an anhydrous basis)	Not more than 50 mg/kg on the dried basis for modified cereal starches Not more than 10 mg/kg on the dried basis for other modified starches
Arsenic	Not more than 1 mg/kg	–
Lead	Not more than 2 mg/kg (on an anhydrous basis)	Not more than 2 mg/kg on the dried basis
Mercury	Not more than 0.1 mg/kg	–
Manganese	–	Not more than 50 mg/kg on the dried basis

3.2.7. Acetylated distarch phosphate (E 1422)

Table 8: Specifications for acetylated distarch adipate (E 1422) according to Commission Regulation (EU) No 231/2012 and JECFA (2016a)

	Commission Regulation (EU) No 231/2012	JECFA (2016a)
Definition	Acetylated distarch adipate is starch cross-linked with adipic anhydride and esterified with acetic anhydride	Acetylated distarch adipate is a modified starch. It is obtained by esterification of food starch with acetic anhydride and esterification/cross-linking with adipic anhydride, in accordance with good manufacturing practice. Acetylation results in substitution of hydroxyl groups with acetyl esters. In cases of cross-linking, where adipic anhydride connects two chains, the structure can be represented by: Starch–O–R–O–Starch, where R = CO–(CH ₂) ₄ –CO and Starch refers to the linear and/or branched structure. Acetylated distarch adipate may additionally be subjected to acid, alkali, enzyme or bleaching treatment in accordance with good manufacturing practice
Description	White or nearly white powder or granules or (if pregelatinised) flakes, amorphous powder or coarse particles	White or nearly white powder or granules or (if pregelatinised) flakes, or amorphous powder or coarse particles
Identification		
Microscopic observation	Passes test (if not pregelatinised)	Passes test
Iodine staining	Passes test (dark blue to light red colour)	Passes test
Solubility	–	Insoluble in cold water (if not pregelatinised); forming typical colloidal solutions with viscous properties in hot water; insoluble in ethanol
Copper reduction	–	Passes test
Specific reaction for acetyl groups	–	Passes test
Ester groups	–	Passes test
Purity		
Loss on drying	Not more than 15.0% for cereal starch Not more than 21.0% for potato starch Not more than 18.0% for other starches	Cereal starch: not more than 15.0% Potato starch: not more than 21.0% Other starches: not more than 18.0% (120°, 4 h, vacuum not exceeding 100 mmHg)
Acetyl groups	Not more than 2.5% (on an anhydrous basis)	Not more than 2.5% on the dried basis

	Commission Regulation (EU) No 231/2012	JECFA (2016a)
Adipate groups	Not more than 0.135% (on an anhydrous basis)	Not more than 0.135% on the dried basis
Carboxyl groups	–	Not more than 0.1% on the dried basis
Sulfur dioxide	Not more than 50 mg/kg for modified cereal starches (on an anhydrous basis) Not more than 10 mg/kg for other modified starches, unless otherwise specified (on an anhydrous basis)	Not more than 50 mg/kg on the dried basis for modified cereal starches Not more than 10 mg/kg on the dried basis for other modified starches
Arsenic	Not more than 1 mg/kg	–
Lead	Not more than 2 mg/kg (on an anhydrous basis)	Not more than 2 mg/kg on the dried basis
Mercury	Not more than 0.1 mg/kg	–
Manganese	–	Not more than 50 mg/kg on the dried basis

3.2.8. Hydroxypropyl starch (E 1440)

Table 9: Specifications for hydroxypropyl starch (E 1440) according to Commission Regulation (EU) No 231/2012 and JECFA (2016a)

	Commission Regulation (EU) No 231/2012	JECFA (2016a)
Definition	Hydroxypropyl starch is starch etherified with propylene oxide	Hydroxypropyl starch is a modified starch. It is obtained by etherification of food starch with propylene oxide, in accordance with good manufacturing practice. Hydroxypropylation results in substitution of hydroxyl groups with 2-hydroxypropyl ether. Hydroxypropyl starch may additionally be subjected to acid, alkali, enzyme or bleaching treatment in accordance with good manufacturing practice
Description	White or nearly white powder or granules or (if pregelatinised) flakes, amorphous powder or coarse particles	White or nearly white powder or granules or (if pregelatinised) flakes, or amorphous powder or coarse particles
Identification		
Solubility	–	Insoluble in cold water (if not pregelatinised); forming typical colloidal solutions with viscous properties in hot water; insoluble in ethanol
Microscopic observation	Passes test (if not pregelatinised)	Passes test
Iodine staining	Passes test (dark blue to light red colour)	Passes test
Copper reduction	–	Passes test
Hydroxypropyl ether groups	–	Passes test
Purity		
Loss on drying	Not more than 15.0% for cereal starch Not more than 21.0% for potato starch Not more than 18.0% for other starches	Cereal starch: not more than 15.0% Potato starch: not more than 21.0% Other starches: not more than 18.0% (120°, 4 h, vacuum not exceeding 100 mmHg)
Hydroxypropyl groups	Not more than 7.0% (on an anhydrous basis)	Not more than 7.0% on the dried basis
Carboxyl groups	–	Not more than 0.1% on the dried basis
Propylene chlorohydrin	Not more than 1 mg/kg (on an anhydrous basis)	Not more than 1 mg/kg

	Commission Regulation (EU) No 231/2012	JECFA (2016a)
Sulfur dioxide	Not more than 50 mg/kg for modified cereal starches (on an anhydrous basis) Not more than 10 mg/kg for other modified starches, unless otherwise specified (on an anhydrous basis)	Not more than 50 mg/kg on the dried basis for modified cereal starches Not more than 10 mg/kg on the dried basis for other modified starches
Arsenic	Not more than 1 mg/kg	–
Lead	Not more than 2 mg/kg (on an anhydrous basis)	Not more than 2 mg/kg on the dried basis
Mercury	Not more than 0.1 mg/kg	–
Manganese	–	Not more than 50 mg/kg on the dried basis

3.2.9. Hydroxypropyl distarch phosphate (E 1442)

Table 10: Specifications for hydroxypropyl distarch phosphate (E 1442) according to Commission Regulation (EU) No 231/2012 and JECFA (2016a)

	Commission Regulation (EU) No 231/2012	JECFA (2016a)
Definition	Hydroxypropyl distarch phosphate is starch cross-linked with sodium trimetaphosphate or phosphorus oxychloride and etherified with propylene oxide	Hydroxypropyl distarch phosphate is a modified starch. It is obtained in accordance with good manufacturing practice by esterification of food starch with sodium trimetaphosphate or phosphorus oxychloride combined with etherification by propylene oxide. Hydroxypropylation results in substitution of hydroxyl groups with 2-hydroxypropyl ether. In cases of cross-linking, where phosphorus oxychloride connects two chains, the structure can be represented by: Starch–O–R–O–Starch, where R = cross-linking group and Starch refers to the linear and/or branched structure. Hydroxypropyl distarch phosphate may additionally be subjected to acid, alkali, enzyme or bleaching treatment in accordance with good manufacturing practice
Description	White or nearly white powder or granules or (if pregelatinised) flakes, amorphous powder or coarse particles	White or nearly white powder or granules or (if pregelatinised) flakes, or amorphous powder or coarse particles
Identification		
Solubility	–	Insoluble in cold water (if not pregelatinised); forming typical colloidal solutions with viscous properties in hot water; insoluble in ethanol
Microscopy	Passes test (if not pregelatinised)	Passes test
Iodine stain	Passes test (dark blue to light red colour)	Passes test
Copper reduction	–	Passes test
Hydroxypropyl ether groups	–	Passes test
Purity		
Loss on drying	Not more than 15.0% for cereal starch Not more than 21.0% for potato starch Not more than 18.0% for other starches	Cereal starch: not more than 15.0% Potato starch: not more than 21.0% Other starches: not more than 18.0% (120°, 4 h, vacuum not exceeding 100 mmHg)
Hydroxypropyl groups	Not more than 7.0% (on an anhydrous basis)	Not more than 7.0% (calculated on dry substance)

	Commission Regulation (EU) No 231/2012	JECFA (2016a)
Carboxyl groups	–	Not more than 0.1% on the dried basis
Propylene chlorohydrin	Not more than 1 mg/kg (on an anhydrous basis)	Not more than 1 mg/kg
Residual phosphate	Not more than 0.14% (as P) for wheat or potato starch (on an anhydrous basis) Not more than 0.04% (as P) for other starches (on an anhydrous basis)	Not more than 0.14% on the dried basis for potato and wheat starch Not more than 0.04% on the dried basis for other starches
Sulfur dioxide	Not more than 50 mg/kg for modified cereal starches (on an anhydrous basis) Not more than 10 mg/kg for other modified starches, unless otherwise specified (on an anhydrous basis)	Not more than 50 mg/kg on the dried basis for modified cereal starches Not more than 10 mg/kg on the dried basis for other modified starches
Arsenic	Not more than 1 mg/kg	–
Lead	Not more than 2 mg/kg (on an anhydrous basis)	Not more than 2 mg/kg on the dried basis
Mercury	Not more than 0.1 mg/kg	–
Manganese	–	Not more than 50 mg/kg on the dried basis

3.2.10. Starch sodium octenyl succinate (E 1450)

Table 11: Specifications for starch sodium octenyl succinate (E 1450) according to Commission Regulation 231/2012 and JECFA (2016a)

	Commission Regulation (EU) No 231/2012	JECFA (2016a)
Definition	Starch sodium octenyl succinate is starch esterified with octenylsuccinic anhydride	Starch sodium octenylsuccinate is a modified starch. It is obtained by esterification of food starch with octenylsuccinic anhydride, and neutralisation with either sodium hydroxide or sodium carbonate as a pH buffer, in accordance with good manufacturing practice. Starch sodium octenylsuccinate may additionally be subjected to acid, alkali, enzyme or bleaching treatment in accordance with good manufacturing practice
Description	White or nearly white powder or granules or (if pregelatinised) flakes, amorphous powder or coarse particles	White or nearly white powder or granules or (if pregelatinised) flakes, or amorphous powder or coarse particles
Identification		
Solubility		Insoluble in cold water (if not pregelatinised); forming typical colloidal solutions with viscous properties in hot water; insoluble in ethanol
Microscopic observation	Passes test (if not pregelatinised)	Passes test
Iodine staining	Passes test (dark blue to light red colour)	Passes test
Copper reduction	–	Passes test
Ester groups	–	Passes test
Purity		
Loss on drying	Not more than 15.0% for cereal starch Not more than 21.0% for potato starch Not more than 18.0% for other starches	Cereal starch: not more than 15.0% Potato starch: not more than 21.0% Other starches: not more than 18.0% (120°, 4 h, vacuum not exceeding 100 mmHg)
Octenylsuccinyl groups	Not more than 3% (on an anhydrous basis)	Not more than 3% on the dried basis

	Commission Regulation (EU) No 231/2012	JECFA (2016a)
Octenylsuccinic acid residue	Not more than 0.3% (on an anhydrous basis)	Not more than 0.3% on the dried basis
Carboxyl groups	–	Not more than 0.1% on the dried basis
Sulfur dioxide	Not more than 50 mg/kg for modified cereal starches (on an anhydrous basis) Not more than 10 mg/kg for other modified starches, unless otherwise specified (on an anhydrous basis)	Not more than 50 mg/kg on the dried basis for modified cereal starches Not more than 10 mg/kg on the dried basis for other modified starches
Arsenic	Not more than 1 mg/kg	–
Lead	Not more than 2 mg/kg (on an anhydrous basis)	Not more than 2 mg/kg on the dried basis
Mercury	Not more than 0.1 mg/kg	–
Manganese	–	Not more than 50 mg/kg on the dried basis

3.2.11. Acetylated oxidised starch (E 1451)

Table 12: Specifications for acetylated oxidised starch (E 1451) according to Commission Regulation (EU) No 231/2012 and JECFA (2016a)

	Commission Regulation (EU) No 231/2012	JECFA (2016a)
Definition	Acetylated oxidised starch is starch treated with sodium hypochlorite followed by esterification with acetic anhydride	Acetylated oxidised starch is a modified starch. It is obtained by treatment of food starch with sodium hypochlorite followed by esterification with acetic anhydride in accordance with good manufacturing practice. Oxidation involves the deliberate production of carboxyl groups. Acetylation results in substitution of hydroxyl groups with acetyl esters. Acetylated oxidised starch may additionally be subjected to acid, alkali, enzyme or bleaching treatment in accordance with good manufacturing practice
Description	White or nearly white powder or granules or (if pregelatinised) flakes, amorphous powder or coarse particles	White or nearly white powder or granules or (if pregelatinised) flakes, or amorphous powder or coarse particles
Identification		
Microscopic observation	Passes test (if not pregelatinised)	Passes test
Iodine staining	Passes test (dark blue to light red colour)	Passes test
Solubility	–	Insoluble in cold water (if not pregelatinised); forming typical colloidal solutions with viscous properties in hot water; insoluble in ethanol
Copper reduction	–	Passes test
Hypochlorite oxidised starch	–	Passes test
Specific reaction for acetyl groups	–	Passes test
Ester groups	–	Passes test
Purity		
Loss on drying	Not more than 15.0% for cereal starch Not more than 21.0% for potato starch Not more than 18.0% for other starches	Cereal starch: not more than 15.0% Potato starch: not more than 21.0% Other starches: not more than 18.0% (120°, 4 h, vacuum not exceeding 100 mmHg)

	Commission Regulation (EU) No 231/2012	JECFA (2016a)
Carboxyl groups	Not more than 1.3% (on an anhydrous basis)	Not more than 1.3% on the dried basis
Acetyl groups	Not more than 2.5% (on an anhydrous basis)	Not more than 2.5% on the dried basis
Sulfur dioxide	Not more than 50 mg/kg for modified cereal starches (on an anhydrous basis) Not more than 10 mg/kg for other modified starches, unless otherwise specified (on an anhydrous basis)	Not more than 50 mg/kg on the dried basis for modified cereal starches Not more than 10 mg/kg on the dried basis for other modified starches
Arsenic	Not more than 1 mg/kg	–
Lead	Not more than 2 mg/kg (on an anhydrous basis)	Not more than 2 mg/kg on the dried basis
Mercury	Not more than 0.1 mg/kg	–
Manganese	–	Not more than 50 mg/kg on the dried basis

3.2.12. Starch aluminium octenyl succinate (E 1452)

Table 13: Specifications for starch aluminium octenyl succinate (E 1452) according to Commission Regulation 231/2012

	Commission Regulation (EU) No 231/2012
Definition	Starch aluminium octenyl succinate is starch esterified with octenylsuccinic anhydride and treated with aluminium sulfate
Description	White or nearly white powder or granules or (if pregelatinised) flakes, amorphous powder or coarse particles
Identification	
Microscopic observation	Passes test (if not pregelatinised)
Iodine staining	Passes test (dark blue to light red colour)
Purity	
Loss on drying	Not more than 21.0%
Octenylsuccinyl groups	Not more than 3% (on an anhydrous basis)
Octenylsuccinic acid residue	Not more than 0.3% (on an anhydrous basis)
Sulfur dioxide	Not more than 50 mg/kg for modified cereal starches (on an anhydrous basis) Not more than 10 mg/kg for other modified starches, unless otherwise specified (on an anhydrous basis)
Arsenic	Not more than 1 mg/kg
Lead	Not more than 2 mg/kg (on an anhydrous basis)
Mercury	Not more than 0.1 mg/kg
Aluminium	Not more than 0.3% (on an anhydrous basis)

No JECFA specification for starch aluminium octenyl succinate (E 1452) is available. According to industry, 'this additive is not commonly marketed in the EU given its limited use as an additive in food supplements; consequently, the EU Starch Industry Association and its members are not aware of any common trade names for food additive use' (Documentation provided to EFSA n. 1).

3.2.13. General remarks on specifications

In addition to the information reported in the tables, the original JECFA monographs contain details on analytical tests to be used for identification purposes and purity determination. However, in most cases, JECFA assigned a tentative status to the specifications, as it was recognised that some critical information was required for setting full specifications and removal of the provisional condition.

The Panel noted that, according to the EU specifications for these modified starches, impurities of the toxic elements arsenic, lead and mercury are accepted up to a concentration of 1, 2 and 0.1 mg/kg.

Contamination at such levels could have a significant impact on the exposure to these metals, for which exposure is already close to the health-based guidance values benchmark doses (lower confidence limits) established by EFSA (EFSA CONTAM Panel (2009, 2010, 2012a). The Panel noted that in JECFA specifications, no limits for arsenic and mercury are available; limits were established only for lead and manganese (JECFA, 2016a).

The Panel further noted that the description of regulatory specifications for modified starches is frequently incomplete and in general not consistent with the format used for other food additives, in that the parameters that permit to differentiate between modified starches should come as part of the assay rather than under purity. Maximum limits for the chemical modifiers linked to starches and chemical residues should be established and presented in the assay and purity sections, respectively. The Panel also noted that it is not clear if the maximum limit for aluminium (0.3%) in E 1452 refers to aluminium linked to the starch or to residual aluminium.

In its report in 2014, JECFA noted that the Eighth Session of the Codex Committee on Contaminants in Foods (CCCF, 2014) agreed to a maximum level (ML) of 0.01 mg/kg for lead in infant formula (as consumed) (JECFA, 2014). The Committee also noted that the use of starch sodium octenyl succinate as a food additive at the proposed use levels could result in an exceedance of the ML of lead in infant formula. This situation was estimated to occur if lead were present in the additive at the specified limit of 2 mg/kg starch sodium octenyl succinate. This estimation was calculated without considering the contribution of other ingredients to the overall lead level in infant formulas. The Committee also noted that the responsibility for ensuring that the final infant formulas comply with the ML for lead remains with infant formula producers. Furthermore, the Committee noted that data provided by the sponsors indicate that the food additive can be produced with lead levels below the limit of 2 mg/kg starch sodium octenyl succinate.

Considering this, the Committee noted that lower lead limits in the specifications, for instance, 0.1 mg/kg for starch sodium octenyl succinate, would not result in the additive exceeding the ML for lead in the final infant formula (i.e. 0.01 mg/kg) (JECFA, 2014).

The Panel noted that there are monographs in the European Pharmacopoeia (European Pharmacopoeia, 2014) on modified starches, 'hydroxypropyl starch' and 'pregelatinised hydroxypropyl starch'. They may be partially hydrolysed using acid or enzyme treatment. In these monographs, limits for total anaerobic microbial count (TAMC) and total combined yeast and mould count (TYMC) are defined, and the absence of *Escherichia coli* and *Salmonella* is required.

According to industry, the analytical data demonstrate that the residual levels of microorganisms are not a safety concern in any of the modified starches. Microbiological contamination is not considered a significant concern for modified starches, as the process steps during the manufacturing process including reactants, pH and high temperatures generate an environment that is not suitable for microbiological survival and growth. While no microbial specifications exist in Commission Regulation (EC) No 231/2012 for any individual modified starch, routine analyses of hazardous microorganisms are conducted by manufacturers to ensure that microbiological contamination is not a concern in the final ingredient. Moreover, microbial contamination is further prevented during the production of modified starches given that all manufacturers have appropriate quality control systems in place (i.e. good hygiene practices (GHP), good manufacturing practices (GMP) and Hazard Analysis Critical Control Point (HACCP) systems) as required under Regulation (EC) No 852/2004¹³ (Documentation provided to EFSA n. 2).

However, the Panel noted that during post-manufacturing handling, microbial contamination of the additives can occur.

The Panel noted that, different from other polysaccharides, no microbiological criteria were defined for modified starches by the EU Regulation. The Panel also noted that the microbiological specifications for polysaccharidic thickening agents, such as starches and gums, should be harmonised, as it is the case for other polysaccharidic thickening agents (e.g. alginic acid and its salts (E 400–E 404), agar (E 406), carrageenan (E 407), processed eucheuma seaweed (E 407a), xanthan gum (E 415), gellan gum (E 418)).

3.3. Manufacturing process

The native starch used for the manufacture of modified starches is obtained from potatoes, cereals (e.g. corn or maize), or other sources (e.g. tapioca) (Documentation provided to EFSA n. 1). Although

¹³ Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. OJ L 139, p. 1–54.

manufacturers can use different specific conditions to produce the modified starches dealt with in this Opinion, the utilised processes reflect the same general scheme: native starch is first subject to chemical modification(s) following treatment with chemical reagent(s) and processing aid(s) as described below; the reaction product is then washed, dewatered, ground and sieved, and packaged. The percent concentrations appearing in the following paragraphs are given on the dry weight of the starch.

3.3.1. Oxidised starch (E 1404)

A slurry of native starch is adjusted to a mildly alkaline pH by addition of a base, and then oxidised using sodium hypochlorite. The desired degree of oxidation is measured by fluidity or viscosity. When the reaction is complete, the slurry is neutralised, the excess of hypochlorite is destroyed by addition of a reducing agent (e.g. bisulfite) and the starch granules are washed, dewatered and dried (Documentation provided to EFSA n. 1; Wurzburg, 2006). As these modified starches are obtained by treatment with hypochlorite, they are also known as 'chlorinated' starches, although no chlorine is present in their structure.

During manufacture of oxidised starch, four possible types of oxidation occur. First, oxidation of primary hydroxyl groups at C-6 position leads to uronic acids. The content of carboxyl groups is restricted by Commission Regulation (EU) No 231/2012 to $\leq 1.1\%$. Secondly, secondary hydroxyls are oxidised to ketone groups. Thirdly, the glucopyranose ring is broken between C-2 and C-3, with subsequent oxidation of the aldehydic to carboxyl groups at C-2 and C-3. Fourthly, oxidation of the end groups plays a minor role (Documentation provided to EFSA n. 1; Wurzburg, 2006). According to JECFA (1975b), oxidised starches contain normally 1% (w/w) of carboxyl and 0.5% (w/w) of keto groups.

3.3.2. Monostarch phosphate (E 1410)

These monosubstituted modified starches are phosphate esters in which one starch hydroxyl is esterified by phosphoric acid to form the monoester. Monostarch phosphate is produced by reaction of orthophosphoric acid, sodium or potassium orthophosphate or sodium tripolyphosphate, with native starch. The production process may be carried out via a wet, semidry or dry process, temperature and pH being important reaction parameters (Documentation provided to EFSA n. 1; Wurzburg, 2006). In the wet process, the phosphating agent is added to an aqueous slurry of native starch, in which temperature and pH are adjusted as necessary, the latter normally being in the 5.5–6.0 range (a low pH can determine hydrolysis of the starch, while a high pH increases the probability of cross-linking and formation of distarch phosphates). In the semidry or dry processes, native starch is mixed with the phosphating agent and heated to ca 120–170°C. The commercial modified starch is obtained by filtration and drying (Documentation provided to EFSA n. 1), as appropriate.

3.3.3. Distarch phosphate (E 1412)

Distarch phosphate is manufactured by treatment of an aqueous slurry of native starch with either sodium trimetaphosphate or phosphorus oxychloride under alkaline conditions. Once cross-linking is achieved, the suspended reacted starch is recovered after neutralisation with acid; the product is subject to thorough washing with water until salt-free, dewatering, and drying (Documentation provided to EFSA n. 1). In the first process, a trimetaphosphate salt is added to an aqueous suspension of starch in the presence of an alkaline catalyst (Kite, 1971); the starch chains are cross-linked at an approximate rate of one phosphate link per 620 glucopyranose units (JECFA, 1974b). In the second process, 0.15–0.25% phosphorus oxychloride is added slowly to a slurry of 40% starch in water at pH 10 and ca 27°C (Kite, 1971); the maximum number of phosphate bridges could reach one per 100 glucopyranose units (JECFA, 1974b). According to Wurzburg (2006), in the preparation of distarch phosphates, treatment is limited to a maximum of 1% of sodium trimetaphosphate or up to 0.1% phosphorus oxychloride.

3.3.4. Phosphated distarch phosphate (E 1413)

Phosphated distarch phosphate has been described as manufactured from high-amylose corn whose starch typically consists of 50–70% amylose and 30–50% amylopectin (Ratnayake and Jackson, 2003; EFSA NDA Panel, 2010). In the production process, an aqueous slurry of native starch is treated with a combination of the reagents permitted for production of monostarch phosphate (E 1410)

(i.e. orthophosphoric acid, sodium or potassium orthophosphate or sodium tripolyphosphate) and of distarch phosphate (E 1412) (sodium trimetaphosphate or phosphorus oxychloride) under alkaline conditions. After the appropriate extent of phosphorylation and cross-linking is achieved, the modified starch is recovered by neutralisation, washing thoroughly with water and drying (Documentation provided to EFSA n. 1).

3.3.5. Acetylated distarch phosphate (E 1414)

Acetylated distarch phosphate has been described as manufactured by treatment of a slurry or suspension of native starch in water with sodium trimetaphosphate or phosphorous oxychloride under alkaline conditions. After the appropriate degree of cross-linking is achieved, the starch is esterified by treatment with either acetic anhydride under mildly alkaline conditions (pH 8–9.5) or with vinyl acetate under alkaline conditions. The modified starch is recovered by neutralisation with acid, washing thoroughly with water, dewatering and drying (Documentation provided to EFSA n. 1). Modification is usually performed by the use of up to 0.1% phosphorus oxychloride and 5% acetic anhydride (JECFA, 1982c). The free and combined phosphate, calculated as phosphorus, must not exceed 0.04% for distarch phosphate made from cereal starch other than wheat and 0.14% for that made from potato and wheat starch (JECFA, 2016a).

3.3.6. Acetylated starch (E 1420)

Acetylated starch is manufactured by treatment of a slurry or suspension of native starch in water with either acetic anhydride under mildly alkaline conditions or vinyl acetate under alkaline conditions. In the first case, esterification is performed with up to 8% acetic anhydride at a pH in the range of 8–9.5 and a temperature of 25–30°C. In the alternative approach, up to 7.5% vinyl acetate is used at a pH in the range of 7.5–12.5 (JECFA, 1982f; Xie et al., 2005; Wurzburg, 2006). After the appropriate degree of esterification is achieved, the modified starch is recovered by neutralisation with acid, washing thoroughly with water, dewatering and drying (Documentation provided to EFSA n. 1).

3.3.7. Acetylated distarch adipate (E 1422)

Acetylated distarch adipate is manufactured by treatment of an aqueous slurry of native starch with a mixture of adipic anhydride and acetic anhydride under mildly alkaline conditions. The reaction is quenched by addition of acid after the appropriate degree of cross-linking and acetylation is reached, and the modified starch is recovered by washing thoroughly with water, dewatering and drying (Documentation provided to EFSA n. 1; JECFA, 1982b; Wurzburg, 2006). According to JECFA (1982b), the number of adipic cross-links does not exceed 1 in about 1,000 glucopyranose units (up to approximately 0.09% adipyl groups).

3.3.8. Hydroxypropyl starch (E 1440)

Hydroxypropyl starch is manufactured by treatment of a slurry or suspension of native starch in water with maximum 10% propylene oxide in the presence of 0.5–1.0% sodium hydroxide. In general, sodium sulfate is added to minimise the gelatinisation or swelling of the starch granules. The reaction is usually carried out at temperatures in the range of 38–52°C for 24 h: below 38°C, the rate of reaction is very slow, while the starch granules may swell at temperatures above 52°C. The resultant starch is usually lightly oxidised, bleached or acid modified after etherification. After the appropriate hydroxypropylation is achieved, the modified starch is neutralised by the addition of acid, washed thoroughly, dewatered and dried (Documentation provided to EFSA n. 1; JECFA, 1982d; Wurzburg, 2006).

3.3.9. Hydroxypropyl distarch phosphate (E 1442)

Hydroxypropyl distarch phosphate is manufactured by treatment of a slurry or suspension of native starch in water with sodium trimetaphosphate or phosphorus chloride under alkaline conditions, followed by etherification with propylene oxide. After the appropriate extent of cross-linking and etherification are achieved, the modified starch is recovered by neutralisation with acid, washing thoroughly with water, dewatering and drying (Documentation provided to EFSA n. 1). According to Wurzburg (2006), in the preparation of distarch phosphates, treatment is limited to a maximum of 1%

of sodium trimetaphosphate or up to 0.1% phosphorus oxychloride. The free and combined phosphate, calculated as phosphorus, must not exceed 0.04% for distarch phosphate made from cereal starch other than wheat and 0.14% for that made from potato and wheat starch (JECFA, 2016a).

3.3.10. Starch sodium octenyl succinate (E 1450)

Starch sodium octenyl succinate is manufactured by treatment of a slurry of native starch in water with octenylsuccinic anhydride under mildly alkaline conditions. Reaction is carried out by slowly adding octenylsuccinic anhydride to a suspension of granular starch while maintaining the pH at 7.0 or higher and stirring vigorously. The maximum DS is approximately one octenyl monosuccinate substituent group for about every 50 anhydroglucose units. After the appropriate extent of esterification is achieved, the modified starch is recovered by neutralisation with acid, washing with water, dewatering and drying (Documentation provided to EFSA n. 1; Wurzburg, 2006).

3.3.11. Acetylated oxidised starch (E 1451)

Acetylated oxidised starch is manufactured in a two-step process. In the first step, an alkaline aqueous slurry is treated with sodium hypochlorite at low temperature (21–38°C). The reaction progress can typically be monitored by following changes in viscosity of the mixture. The excess of hypochlorite is destroyed by adding sodium bisulfite, and the resulting organic salts are removed by washing with water. In the second step, the oxidised starch is then esterified with acetic anhydride under mildly alkaline conditions. The resulting product is recovered by neutralisation with acid, washing thoroughly with water, dewatering and drying (Documentation provided to EFSA n. 1; JECFA, 2002b).

3.3.12. Starch aluminium octenyl succinate (E 1452)

Starch aluminium octenyl succinate is manufactured by treatment of a slurry of native starch in water with maximum 3% octenylsuccinic anhydride under mildly alkaline conditions (Documentation provided to EFSA n. 1; Wurzburg, 2006). According to Nair and Yamarik (2002), when the appropriate extent of esterification is achieved, the mixture is treated with an amount of aluminium sulfate, not exceeding 2% of the starch weight. The modified starch is recovered by neutralisation with acid, washing with water, dewatering and drying (Documentation provided to EFSA n. 1).

3.4. Methods of analysis

According to the European Starch Industry Association (Association des Amidonniers et Féculiers (AAF)) (Documentation provided to EFSA n. 1), there are no validated methods available for the identification of modified starches in foodstuffs for a number of reasons, amongst which:

- 'whilst a product specific identification method might in theory be possible, given the diverse composition of food matrices to which modified starches are added (egg products, beverages, bakery products, etc.), a general protocol of practical use is not feasible;
- similarly, specific identification methods based on the modification of the starch might in theory be possible but in a practical sense, the DS is relatively low and the modified starches are often used in combination in foodstuffs, making development of a general protocol not feasible.'

Methods of analysis that might be applicable to detect modified starch identifiers could typically be based on the analysis of specific compositional parameters (e.g. the content of phosphorous and aluminium and of carboxyl, acetyl, adipate, hydroxypropyl and octenylsuccinyl groups) (JECFA, 2016a). According to the conclusions of the 82nd JECFA meeting (JECFA, 2016b), missing data were identified that are required by JECFA to define specifications for a number of modified starches: these data include, among others, the availability of suitable tests to identify the phosphate, adipate and octenyl succinate groups, propylene chlorohydrin, and to evaluate cross-linking.

What follows are relevant examples of methods developed in the 1980s and 1990s for the analysis of starches and modified starches in foods: at that time, methods to analyse starches with enzymatic treatments were already taking over the commonly used chemical methods due to a lack of specificity of the latter and their dependence on corrosive and dangerous reagents (Karkalas, 1985).

A method for the analysis of starch and chemically modified starches in different foods was published by Karkalas (1985). The method was based on sequential hydrolysis with thermostable

bacterial α -amylase and fungal amyloglucosidase; the formed glucose – ultimate product of starch hydrolysis – was determined colorimetrically by a glucose oxidase–peroxidase–chromogen system at pH 7. Native normal and waxy starches, and distarch phosphate, gave quantitative yields of glucose with a high degree of precision (coefficient of variation, < 1%); oxidised starch did not yield glucose quantitatively due to the presence of dicarboxylic groups in the polymer. Acetylated distarch phosphate, high-amylose starch and retrograded amylose were initially treated with 1M NaOH for 30 min, then neutralised and analysed as normal starch. After neutralisation, α -amylase was added to hydrolyse starch to glucose. Interfering oligosaccharides were removed by a previous treatment with 75% (v/v) 2-propanol, an extraction technique adequate for various types of samples. In conclusion, the Karkalas' method yielded quantitative recoveries of starch from normal and waxy varieties; quantitative recoveries were also obtained from acetylated distarch phosphate and retrograde amylose after alkaline pretreatment and neutralisation. The method was found to be accurate, reproducible and rapid, and useful to determine the starch content in foods; however, it could not distinguish between native starch and its different modifications.

Chatel et al. (1996) provided an example of starch identification and determination in sweetened food products such as sweetened fruit preparations. Dialysis was used as a purification technique that allowed the preservation of starch while simultaneously eliminating sucrose. The study of experimental parameters such as dialysis membrane cut-off, treatment time, temperature and water renewal indicated a yield of sucrose elimination of 99.4% (relative standard deviation (RSD) 0.1%) for strawberry preparations. Enzymatic hydrolysis of starch was carried out by α -amylase and amyloglucosidase. Previous gelatinisation–alkalinisation treatment, starch modification, hydrolysis time and pH were observed to have a considerable effect on the efficacy of hydrolysis. The glucose released by hydrolysis was determined with hexokinase and glucose-6-phosphate dehydrogenase. The use of this method in strawberry preparations containing 3.0% (w/w) acetylated distarch adipate led to the determination of 92% (RSD, 3.1%) starch. The method was considered to be efficient, specific and reproducible for the samples analysed.

In a subsequent paper, Chatel et al. (1997) optimised the dialysis and gelatinisation steps and the infrared (IR) identification of starch chemical modifications. The study was set out in recognition that identification and quantification of starch used as a thickening and gelling agent in sweetened fruit preparations were difficult due to the low levels of concentration and the heterogeneous, gelified and highly sweetened nature of the medium. In a first step, starches were identified by optical microscopy; acetyl and hydroxypropyl modifications were characterised by Fourier transform IR spectroscopy (FT-IR). For quantification, starch was first extensively purified by dialysis to remove most (> 99%) of the sucrose. The experimental conditions of this step were optimised. A previous gelatinisation treatment improved significantly the enzymatic hydrolysis of starch and the quantification of the released glucose. The method developed, applied to 15 different sweetened fruit preparations, allowed reliable determinations of distarch phosphate, acetylated distarch phosphate and acetylated distarch adipate. According to the authors, it also provided an appropriate tool for the special case of a hydroxypropylated distarch phosphate.

3.5. Reaction and fate in food

For all the modified starches dealt with in this opinion, AAF recommends a storage time not longer than 2 years (Documentation provided to EFSA n. 1), as the moisture content may gradually increase to the extent of impairing starch physical properties (Lloyd and Kirst, 1963). In practice, the typical shelf-life is 5 years under appropriate storage conditions, which include a dry place at room temperature and the original packaging kept unopened (Documentation provided to EFSA n. 1). The stability of modified starches – as indicated by the lack of known relevant degradation or reaction products and acknowledged on the basis of a vast patrimony of commercial data – is supported by history of use (over 20 years) and the chemical nature of the additives. Stability is further supported by:

- chemical modifications and their influence on the properties of the material are clearly understood;
- the extent of chemical modification is set by legislation to reflect the maximum treatment necessary to achieve the desired properties in food applications, and is generally low (cfr. Commission Regulation (EU) 231/2012 laying down specifications for food additives);
- stability is indirectly demonstrated by the retention of effect under the conditions of use in different foods.

As reported by AAF (Documentation provided to EFSA n. 1), native starches have useful applications as food additives; however, their use has a number of limitations reflecting their chemical and physical properties, like poor solubility and high hydrophilicity, which in fact restrict their use. In modified starches, the chemical and physical characteristics of the native substances are altered in order to improve the functional properties for particular food applications: the observed effects on such properties depend on the type and extent of the modification (e.g. DS) and the source starch (e.g. cereal, potato, tapioca). The grain size of the starch also affects its reactivity; the larger the grains are, the higher the modification susceptibility is (Lewicka et al., 2015). As a general rule, the extent of modification required to distinctly alter the functional characteristics of native starches is low, as observed above with reference to the limitations imposed by Commission Regulation (EU) No 231/2012. In conclusion, for a large number of food applications, modified starches are used because of their superior properties compared to the native substances, a feature touched upon by many authors as, for instance, may be inferred from Aćkar et al. (2015), Committee on Nutrition (1978), FASEB (1979), Fortuna et al. (1992), JECFA (2002a), Khalil et al. (1995), Kite (1971), Liu et al. (1999), and references cited therein.

It can be observed that modified starches are not only a product of modifications due to chemical reactions but can also be obtained by making native starches undergo physical modification in order to weaken or degrade the crystalline structure responsible for the integrity of starch granules (Documentation provided to EFSA n. 1; Xie et al., 2005). Contrary to native starches, these physically modified starches will swell in cold or lukewarm water thereby avoiding the need to cook the starch. Chemically modified starches may also be subject to physical modifications.

The rheological properties of starch pastes, as viscosity, are very important for their applications as thickeners or as gelling agents. Acetylated starch is characterised by lower pasting temperature in comparison to the native one (Berski et al., 2011).

Gelatinisation is one of the most important functional properties of starch and microwave processing is able to change the gelatinisation mechanism (Lewicka et al., 2015). The microwave effect on structural changes of the starch leads to a reduction of its viscosity (Lewandowicz et al., 1998).

Sun et al. (2016), studied in a simulation yoghurt environment, the interactions of starches (phosphate starch, hydroxypropyl starch and starch ester of octenyl succinate) and casein, demonstrating that these interactions included electrostatic adhesion, steric stabilisation and hydrogen bond. They found that the interactions of modified starches and casein had a lot of differences with respect to native starches. It was reported that phosphate starch interacted with casein closely due to electrostatic adhesion, while in the hydroxypropyl starch/casein gel the interactions were also due to hydrogen bond and steric stabilisation. Starch ester of octenyl succinate being both hydrophilic and hydrophobic, was adsorbed with casein by steric stabilisation, increasing the structure tightness.

3.6. Authorised uses and use levels

Maximum levels of modified starches have been defined in Annex II to Regulation (EC) No 1333/2008 on food additives, as amended. In this document, these levels are referred to as MPLs.

Currently, 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) and acetylated oxidised starch (E 1451) are authorised food additives in the EU at *quantum satis* (QS) in almost all foods apart from processed cereal-based foods and baby foods for infants and young children and other foods for young children (50,000 mg/kg) and dietary foods for infants, babies and young children for special medical purposes and special formulae for infants (50,000 mg/kg and 20,000 mg/kg for E 1450). These modified starches are included in Group I (food additives authorised at QS).

Table 14 summarises foods that are permitted to contain the modified starches evaluated in the present opinion and the corresponding MPLs as set by Annex II to Regulation (EC) No 1333/2008.

Table 14: MPLs of modified starches (E 1404, E 1410, E 1412, E 1413, E 1414, E 1420, E 1422, E 1440, E 1442, E 1450 and E 1451) in foods according to Annex II to Regulation (EC) No 1333/2008

Food category number	Food category name	E-number/group	Restrictions/exceptions	MPL (mg/L or mg/kg as appropriate)
01.3	Unflavoured fermented milk products, heat-treated after fermentation	Group I		QS
01.4	Flavoured fermented milk products including heat-treated products	Group I		QS
01.6.2	Unflavoured live fermented cream products and substitute products with a fat content of less than 20%	E 1404, E 1410, E 1412, E 1413, E 1414, E 1420, E 1422, E 1440, E 1442, E 1450, E 1451		QS
01.6.3	Other creams	Group I		QS
01.7.1	Unripened cheese excluding products falling in category 16	Group I	Except mozzarella	QS
01.7.5	Processed cheese	Group I		QS
01.7.6	Cheese products (excluding products falling in category 16)	Group I		QS
01.8	Dairy analogues, including beverage whiteners	Group I		QS
02.2.2	Other fat and oil emulsions including spreads as defined by Council Regulation (EC) No 1234/2007 and liquid emulsions	Group I		QS
02.3	Vegetable oil pan spray	Group I		QS
03	Edible ices	Group I		QS
04.2.1	Dried fruit and vegetables	Group I		QS
04.2.2	Fruit and vegetables in vinegar, oil, or brine	Group I		QS
04.2.4.1	Fruit and vegetable preparations excluding compote	Group I		QS
04.2.5.4	Nut butters and nut spreads	Group I		QS
04.2.6	Processed potato products	Group I		QS
05.1	Cocoa and Chocolate products as covered by Directive 2000/36/EC	Group I	Only energy-reduced or with no added sugar	QS
05.2	Other confectionery including breath freshening microsweets	Group I		QS
05.3	Chewing gum	Group I		QS
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	Group I		QS
06.2.2	Starches	Group I		QS
06.3	Breakfast cereals	Group I		QS
06.4.2	Dry pasta	Group I	Only gluten free and/or pasta intended for hypoproteic diets in accordance with Directive 2009/39/EC	QS

Food category number	Food category name	E-number/group	Restrictions/exceptions	MPL (mg/L or mg/kg as appropriate)
06.4.4	Potato gnocchi	Group I	Except fresh refrigerated potato gnocchi	QS
06.4.5	Fillings of stuffed pasta (ravioli and similar)	Group I		QS
06.5	Noodles	Group I		QS
06.6	Batters	Group I		QS
06.7	Pre-cooked or processed cereals	Group I		QS
07.1	Bread and rolls	Group I	Except products in 7.1.1 and 7.1.2	QS
07.2	Fine bakery wares	Group I		QS
08.3.1	Non-heat-treated meat products	Group I		QS
08.3.2	Heat- treated meat products	Group I	Except <i>foie gras</i> , <i>foie gras entier</i> , <i>blocs de foie gras</i> , <i>Libamáj</i> , <i>libamáj egészben</i> , <i>libamáj tömbben</i>	QS
08.3.3	Casings and coatings and decorations for meat	Group I		QS
09.2	Processed fish and fishery products including molluscs and crustaceans	Group I		QS
09.3	Fish roe	Group I	Only processed fish roe	QS
10.2	Processed eggs and egg products	Group I		QS
11.2	Other sugars and syrups	Group I		QS
12.1.2	Salt substitutes	Group I		QS
12.2.2	Seasonings and condiments	Group I		QS
12.3	Vinegars	Group I		QS
12.4	Mustard	Group I		QS
12.5	Soups and broths	Group I		QS
12.6	Sauces	Group I		QS
12.7	Salads and savoury-based sandwich spreads	Group I		QS
12.8	Yeast and yeast products	Group I		QS
12.9	Protein products, excluding products covered in category 1.8	Group I		QS
13.1.3	Processed cereal-based foods and baby foods for infants and young children as defined by Directive 2006/125/EC	E 1404, E 1410, E 1412, E 1413, E 1414, E 1420, E 1422, E 1450, E 1451	Only processed cereal-based foods and baby foods	50,000 ^(a)
13.1.4	Other foods for young children	E 1404, E 1410, E 1412, E 1413, E 1414, E 1420, E 1422, E 1450		50,000 ^(a)
13.1.5.1	Dietary foods for infants for special medical purposes and special formulae for infants	E 1450	Only in infant formulae and follow-on formulae	20,000 ^(a)

Food category number	Food category name	E-number/group	Restrictions/exceptions	MPL (mg/L or mg/kg as appropriate)
13.1.5.2	Dietary foods for babies and young children for special medical purposes as defined in Directive 1999/21/EC	E 1404, E 1410, E 1412, E 1413, E 1414, E 1420, E 1422, E 1451		50,000 ^(a)
13.1.5.2	Dietary foods for babies and young children for special medical purposes as defined in Directive 1999/21/EC	E 1450		20,000 ^(a)
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)	Group I		QS
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)	Group I		QS
13.4	Foods suitable for people intolerant to gluten as defined by Regulation (EC) No 41/2009	Group I	Including dry pasta	QS
14.1.2	Fruit juices as defined by Directive 2001/112/EC and vegetable juices	Group I	Only vegetable juices	QS
14.1.3	Fruit nectars as defined by Directive 2001/112/EC and vegetable nectars and similar products	Group I	Only vegetable nectars	QS
14.1.4	Flavoured drinks	Group I		QS
14.1.5.2	Other	Group I	Excluding unflavoured leaf tea; including flavoured instant coffee	QS
14.2.3	Cider and perry	Group I		QS
14.2.4	Fruit wine and made wine	Group I		QS
14.2.5	Mead	Group I		QS
14.2.6	Spirit drinks as defined in Regulation (EC) No 110/2008	Group I	Except whisky or whiskey	QS
14.2.7.1	Aromatised wines	Group I		QS
14.2.7.2	Aromatised wine-based drinks	Group I		QS
14.2.7.3	Aromatised wine-product cocktails	Group I		QS
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15% of alcohol	Group I		QS
15.1	Potato-, cereal-, flour- or starch-based snacks	Group I		QS
15.2	Processed nuts	Group I		QS
16	Desserts excluding products covered in category 1, 3 and 4	Group I		QS
17.1^(b)	Food supplements supplied in a solid form including capsules and tablets and similar forms, excluding chewable forms	Group I		QS
17.2^(b)	Food supplements supplied in a liquid form	Group I		QS
17.3^(b)	Food supplements supplied in a syrup-type or chewable form	Group I		QS

Food category number	Food category name	E-number/group	Restrictions/exceptions	MPL (mg/L or mg/kg as appropriate)
18	Processed foods not covered by categories 1–17, excluding foods for infants and young children	Group I		QS

MPL: maximum permitted level; QS: *quantum satis*; FCS: Food Categorisation System (food nomenclature presented in Annex II to Regulation (EC) No 1333/2008).

(a): The maximum levels of use indicated refer to foods ready for consumption prepared following manufacturers' instructions.

E 1450 shall be used in conformity with the limits set in the Annexes to Directive 2006/141/EC.

(b): FCS 17 refers to food supplements as defined in Directive 2002/46/EC of the European Parliament and of the Council excluding food supplements for infants and young children.

According to Annex III, Part 1, of Regulation (EC) No 1333/2008, 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) and acetylated oxidised starch (E 1451) are authorised as carriers in all food additives at QS.

In addition, according to Annex III, Part 3 of Regulation (EC) No 1333/2008, E 1404, E 1410, E 1412, E 1413, E 1414, E 1420, E 1422, E 1440, E 1442, E 1450 and E 1451 are also authorised as food additives (including carriers) in food enzymes at QS.

Furthermore, according to Annex III, Part 5, Section A, of Regulation (EC) No 1333/2008, E 1404, E 1410, E 1412, E 1413, E 1414, E 1420, E 1422, E 1440, E 1442, E 1450 and E 1451 are also authorised as food additives (including carriers) in all nutrients at QS, except for nutrients intended to be used in foodstuffs for infants and young children listed in point 13.1 of Part E of Annex II.

However, according to Annex III, Part 5, Section B, E 1420 and E 1451 are authorised as food additive in all nutrients intended to be used in processed cereal based foods and baby foods for infants and young children as defined by Directive 2006/125/EC, under the condition that the maximum level in foods mentioned in point 13.1.3 of Part E of Annex II is not exceeded. Additionally, starch sodium octenyl succinate (E 1450) is authorised as a food additive in vitamin preparations and in polyunsaturated fatty acid preparations intended to be used in foods for infants and young children. When E 1450 is added in nutrients intended to be used in foodstuffs for infants and young children under FCS 13.1, maximum levels of carry-over are 100 mg/kg for vitamin preparations and 1,000 mg/kg for polyunsaturated fatty acid preparations.

Starch aluminium octenyl succinate (E 1452)

Starch aluminium octenyl succinate (E 1452) is an authorised food additive in the EU according to Annex III, Part 5, Section A to Regulation (EC) No 1333/2008 on food additives. Starch aluminium octenyl succinate (E 1452) is authorised for use only as a food additive and carrier in food supplements as defined in Directive 2002/46/EC due to its use in vitamin preparations for encapsulation purposes. The maximum level permitted in the final food is 35,000 mg/kg.

3.7. Exposure data

3.7.1. Reported use levels

Most food additives in the EU are authorised at a specific MPL. However, a food additive may be used at a lower level than the MPL. Therefore, information on actual use levels is required for performing a more realistic exposure assessment, especially for those food additives for which no MPL is set and which are authorised according to QS.

In the framework of Regulation (EC) No 1333/2008 on food additives and of Commission Regulation (EU) No 257/2010 regarding the re-evaluation of approved food additives, EFSA issued a public call¹⁴ in 2010 for occurrence data (usage level and/or concentration data) on modified starches (E 1404, E 1410, E 1412, E 1413, E 1414, E 1420, E 1422, E 1440, E 1442, E 1450, E 1451 and E 1452). In response to this call, some information became available for 11 modified starches (E 1404–E 1450), to be used in combination (Documentation provided to EFSA n. 1). The usage data

¹⁴ Call for scientific data on miscellaneous food additives permitted in the EU and belonging to several functional classes. Published: 8 June 2010. Available online: <http://www.efsa.europa.eu/en/dataclosed/call/ans100609>

provided were representing use level ranges for any of these starches (i.e. not for a specific starch) and therefore could not be used in the present exposure assessment. Some additional information was made available to EFSA on E 1422 and E 1450, which was not taken into account in the assessment, as more up to date information was available (Documentation provided to EFSA n. 3).

In October 2015, a public call¹⁵ for food additive usage level and/or concentration data in food and beverages intended for human consumption, including modified starches, was launched, with a deadline in May 2016. In response to this public call, updated information on the actual use levels of 11 modified starches (E 1404, E 1410, E 1412, E 1413, E 1414, E 1420, E 1422, E 1440, E 1442, E 1450 and E 1451) in foods was made available to EFSA by industry and Member States.

In addition, a public call¹⁶ for usage level data of starch aluminium octenyl succinate (E 1452) has been launched in October 2016. Some information on the use of E 1452 in combinations of vitamin and mineral supplements has been provided to EFSA by industry.

Information on the levels of starch aluminium octenyl succinate (E 1452) in combination with sodium aluminium silicate (E 554) in raw materials used for the production of food supplements were also provided, but these data were not used in the present assessment, as the proportion of their presence in the final products was not specified.

3.7.1.1. Summarised data on reported use levels in foods provided by industry

In total, industry provided EFSA with data on use levels (n = 873) of modified starches (E 1404, E 1410, E 1412, E 1413, E 1414, E 1420, E 1422, E 1440, E 1442, E 1450, E 1451 and E 1452) in foods.

Information on the use levels of modified starches in foods was made available to EFSA by Krueger GmbH & Co., the Association of the European Self-Medication Industry (AESGP), Aviko, the European Dairy Association (EDA), FoodDrinkEurope (FDE), the International Chewing Gum Association (ICGA), Specialised Nutrition Europe (SNE) and Food Supplements Europe (FSE) (Documentation provided to EFSA n. 3–10).

The Panel noted that 67 usage levels provided for the food categories of other creams (FC 1.6.3), decorations, coatings and fillings (FC 5.4), fine bakery wares (FC 7.2), flavoured drinks (FC 14.1.4), desserts (FC 16), dietary foods for infants for special medical purposes and special formulae for infants (FC 13.1.5.1), dietary foods for babies and young children for special medical purposes (FC 13.1.5.2), dietary foods for special medical purposes (FC 13.2) and dietary food for weight control diets (FC 13.3) referred to niche product(s). Since other usage levels were available for all the aforementioned food categories, the Panel decided to exclude these niche product usage levels from further analysis in the refined scenarios, except for dietary foods for infants for special medical purposes and special formulae for infants (FC 13.1.5.1) and dietary foods for babies and young children for special medical purposes (FC 13.1.5.2), where only data from niche products were available.

The number of usage data provided by industry for each modified starch and the number of food categories for which usage data were provided, out of the total authorised food categories are presented in Table 15.

Table 15: Number of usage data provided by industry for each modified starch and number of food categories for which usage data were provided, out of the total authorised food categories

	E-number											
	E 1404	E 1410	E 1412	E 1413	E 1414	E 1420	E 1422	E 1440	E 1442	E 1450	E 1451	E 1452
Usage level data (n)	9	2	53	2	26	57	275	4	116	307	15	7
Number of food categories	9	2	11	2	8	14	26	3	16	23	4	1
Authorised food categories	71	71	71	71	71	71	71	68	68	72	70	1

¹⁵ Call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Published: 12 October 2015. Available online: <https://www.efsa.europa.eu/en/data/call/151012>

¹⁶ Call for usage level data on starch aluminium octenyl succinate (E 1452) in food intended for human consumption. Published: 20 October 2016. Available at: <https://www.efsa.europa.eu/it/data/call/161020>

Appendix A provides data on the use levels of modified starches in foods as reported by industry. Where the usage levels were given on the basis of the powdered (dehydrated) product and not of the final (ready-to-consume) food, dilution factors were applied for the risk assessment.

The wide range of reported usage levels has been justified by the data providers with the fact that the reported values represented several products within each category (i.e. milk-based ice cream and non-milk based ice cream, etc.). Very low values were reported when the additive was present in one minor ingredient of the final product.

3.7.2. Summarised data extracted from the Mintel's Global New Products Database

The Mintel Global New Products Database (GNPD) is an online database which monitors product introductions in consumer packaged goods markets worldwide. It contains information of over 2 million food and beverage products, of which more than 900,000 are or have been available on the European food market. Mintel started covering European Union's food markets in 1996, currently having 20 out of its 28 member countries and Norway presented in the Mintel GNPD.¹⁷

For the purpose of this scientific opinion, the Mintel GNPD¹⁸ was used for checking the labelling of products containing modified starches within the EU's food products, as the Mintel GNPD shows the compulsory ingredient information presented in the labelling of products.

It should be noted that, according to Regulation (EU) No 1169/2011¹⁹, for foodstuffs containing modified starches it is not obligatory to report on the food product label the exact E number; only the term 'modified starches' may be used on the label instead. Taking this into account, it was noted that according to the Mintel GNPD, the food products reported to contain 'modified starches' (E number unspecified) are much more than the products reported to contain these food additives labelled with the respective E number.

Information on both 'modified starches'-labelled and specific E-number-labelled food products is presented in Appendix B. Appendix B presents the number and percentage of the food products labelled between January 2011 and September 2016, out of the total number of food products, per food subcategory, according to the Mintel GNPD food classification.

The Mintel database does not contain any products labelled with monostarch phosphate (E 1410) or phosphated distarch phosphate (E 1413) in the given period. However, their use cannot be excluded taken into account the high number of products where only unspecified 'modified starches' is indicated on the label.

For E 1452, two products (grated cheese and ready-to-eat salad) were identified in the Mintel database labelled with this specific E-number; however, the use of E 1452 is not authorised in these foodstuffs.

The overall percentage of food products labelled with the term 'modified starches' (E number unspecified) considering all relevant food products available (n = 463,158) in Mintel by the time of the query was 8.73% (n = 40,569). However, only 0.53% (n = 2,444) of these products were labelled with the exact E number of the given modified starch.

Considering the Mintel subcategories, the highest percentage of products labelled with the term 'modified starches' was identified for soft cheese desserts (57.75%; 927 out of 1,588 products), chilled desserts (41.24%; 2,681 out of 6,501 products) and mayonnaise (36.92%; 353 out of 956 products). The highest number of products labelled with the term 'modified starches' was found in the subcategories of prepared meals (n = 3,336), spoonable yogurt (n = 3,296) and chilled desserts (n = 2,681).

According to Mintel, modified starches are also used in products of the following food categories, however no usage levels were provided for these food categories by the food industry:

- 06.3 Breakfast cereals
- 06.7 Pre-cooked or processed cereals
- 09.2 Processed fish and fishery products
- 10.2 Processed eggs and egg products
- 11.2 Other sugars and syrups

¹⁷ Missing Bulgaria, Cyprus, Estonia, Latvia, Lithuania, Luxembourg, Malta and Slovenia.

¹⁸ <http://www.gnpd.com/sinatra/home/> accessed on 23/11/2016.

¹⁹ Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers. OJ L 304, p. 18–63.

- 12.7 Salads and savoury-based sandwich spreads
- 12.9 Protein products
- 14.1.2 Fruit juices (only vegetable juices)
- 14.1.3 Fruit nectars (only vegetable nectars)
- 14.2.6 Spirit drinks
- 14.2.7.3 Aromatised wine-product cocktails
- 14.2.8 Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15% alcohol
- 15.2 Processed nuts

This may result in an underestimation of the exposure.

3.7.3. Food consumption data used for exposure assessment

3.7.3.1. EFSA Comprehensive European Food Consumption Database

Since 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) has been populated with national data on food consumption at a detailed level. Competent authorities in the European countries provide EFSA with data on the level of food consumption by the individual consumer from the most recent national dietary survey in their country (cf. Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011a). New consumption surveys added in the Comprehensive database were also taken into account in this assessment.¹²

The food consumption data gathered by EFSA were collected by different methodologies and thus direct country-to-country comparisons should be interpreted with caution. Depending on the food category and the level of detail used for exposure calculations, uncertainties could be introduced owing to possible subjects' underreporting and/or misreporting of the consumption amounts. Nevertheless, the EFSA Comprehensive Database represents the best available source of food consumption data across Europe at present.

Food consumption data from the following population groups: infants, toddlers, children, adolescents, adults and the elderly were used for the exposure assessment. For the present assessment, food consumption data were available from 33 different dietary surveys carried out in 19 European countries (Table 16).

Table 16: Population groups considered for the exposure estimates of modified starches

Population	Age range	Countries with food consumption surveys covering more than one day
Infants	From more than 12 weeks up to and including 11 months of age	Bulgaria, Denmark, Finland, Germany, Italy, UK
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Denmark, Finland, Germany, Italy, Netherlands, Spain, UK
Children ^(a)	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Spain, Sweden, UK
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Italy, Latvia, Spain, Sweden, UK
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Romania, Spain, Sweden, UK
The elderly ^(a)	From 65 years of age and older	Austria, Belgium, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Romania, Sweden, UK

(a): The terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge of 'elderly' and 'very elderly' in the Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011a).

Consumption records were codified according to the FoodEx classification system (EFSA, 2011b). Nomenclature from the FoodEx classification system has been linked to the food categorisation system (FCS) as presented in Annex II to Regulation (EC) No 1333/2008, part D, to perform exposure estimates. In practice, FoodEx food codes were matched to the FCS food categories.

3.7.3.2. Food categories selected for the exposure assessment of modified starches

The food categories in which the use of modified starches is authorised were selected from the nomenclature of the EFSA Comprehensive Database (FoodEx classification system), at the most detailed level possible (up to FoodEx Level 4) (EFSA, 2011b).

Some food categories are not referenced in the EFSA Comprehensive Database and could therefore not be taken into account in the present estimate. This was the case for 10 food categories (Appendix C) and may have resulted in an underestimation of the exposure. However, no usage levels were received for these food categories either. The food categories which were not taken into account are described below (in ascending order of the FCS codes):

- 01.7.6 Cheese products (excluding products falling in category 16); however, these products were reclassified under 01.7.5 Processed cheese
- 02.3 Vegetable oil pan spray
- 06.6 Batters
- 06.7 Pre-cooked or processed cereals
- 08.3.3 Casings and coatings and decorations for meat
- 12.1.2 Salt substitutes
- 14.1.3 Fruit nectars as defined by Directive 2001/112/EC and vegetable nectars and similar products, only vegetable nectars
- 14.2.5 Mead
- 14.2.7.2 Aromatised wine-based drinks
- 14.2.7.3 Aromatised wine-product cocktails

For the following food categories, the restrictions/exceptions which apply to the use of modified starches could not be taken into account, and therefore the whole food category was considered in the exposure assessment. This applies to three food categories (Appendix C) and may have resulted in an overestimation of the exposure:

- 05.1 Cocoa and Chocolate products as covered by Directive 2000/36/EC, only energy-reduced or with no added sugar
- 14.1.5.2 Other, excluding unflavoured leaf tea; including flavoured instant coffee
- 17.1/17.2/17.3 Food supplements, in solid, liquid, syrup-type or chewable form.

According to Annex III, Part 5 to Regulation (EC) No 1333/2008 on food additives, starch aluminium octenyl succinate (E 1452) is authorised for use only as a food additive and carrier in food supplements as defined in Directive 2002/46/EC due to its use in vitamin preparations for encapsulation purposes; thus, considering the whole food category 17 results an overestimation in the exposure.

As regards FC 01.3 Unflavoured fermented milk products, heat-treated after fermentation, differentiating this food category from food category 01.2 Unflavoured fermented milk products, including natural unflavoured buttermilk (excluding sterilised buttermilk) non-heat treated after fermentation, in the Comprehensive database is not possible, therefore these categories were considered together in the estimation.

Similarly, for the food category 01.6.2 Unflavoured live fermented cream products and substitute products with a fat content of less than 20%, the whole food category 01.6 was taken into account in the assessment, as it is not possible to differentiate between FC 01.6.2 and FC 01.6.3 Other creams.

Considering that the food category 18 (Processed foods not covered by categories 1–17, excluding foods for infants and young children) is extremely unspecific (e.g. composite foods), processed foods, prepared or composite dishes belonging to the food category 18 were reclassified under food categories in accordance to their main component. Therefore, food category 18 is not taken into account as contributor to the total exposure estimates.

For all scenarios, four food categories were included in the exposure assessment without considering the restrictions/exceptions as set in Annex II to Regulation (EC) No 1333/2008. Ten food categories were not taken into account in the exposure assessment because these are not referenced in the EFSA Comprehensive Database. Thirty-six food categories were not taken into account because

no concentration data were provided (Appendix C). For the remaining food categories, the refinements considering the restrictions/exceptions as set in Annex II to Regulation No 1333/2008 were applied. Overall, for the maximum level exposure scenario and for the refined scenarios, 31 food categories were included in the present exposure assessment to modified starches.

3.8. Exposure estimates

3.8.1. Exposure to modified starches from their use as food additives

The Panel estimated chronic exposure to modified starches for the following population groups: infants, toddlers, children, adolescents, adults and the elderly.

A combined dietary exposure estimate was calculated for the modified starches E 1404, E 1410, E 1412, E 1413, E 1414, E 1420, E 1422, E 1440, E 1442, E 1450 and E 1451 by selecting, within each food category, the highest use level among the use levels reported for each of the different E-numbers.

A separate scenario was carried out for starch aluminium octenyl succinate (E 1452) which is authorised in EU as a food additive and carrier only in food supplements as defined in Directive 2002/46/EC due to its use in vitamin preparations for encapsulation purposes according to Annex III, Part 5 to Regulation (EC) No 1333/2008 on food additives. Furthermore, exposure to aluminium from E 1452 was also estimated for food supplement consumers.

Dietary exposure to modified starches was calculated by multiplying modified starches concentrations for each food category (Appendix C) with their respective consumption amount per kilogram of body weight for each individual in the Comprehensive Database. The exposure per food category was subsequently added to derive an individual total exposure per day. These exposure estimates were averaged over the number of survey days, resulting in an individual average exposure per day for the survey period. Dietary surveys with only one day per subject were excluded, as they are considered as not adequate to assess repeated exposure.

This was carried out for all individuals per survey and per population group, resulting in distributions of individual exposure per survey and population group (Table 16). On the basis of these distributions, the mean and 95th percentile of exposure were calculated per survey and per population group. The 95th percentile of exposure was only calculated for those population groups where the sample size was sufficiently large to allow this calculation (EFSA, 2011a). Therefore, in the present assessment, 95th percentile of exposure for infants from Italy and for toddlers from Belgium, Italy and Spain were not included.

Exposure assessment to modified starches was carried out by the ANS Panel based on two different sets of concentration data: (1) maximum reported use levels provided to EFSA (defined as the *maximum level exposure assessment scenario*) and, (2) reported use levels (defined as the *refined exposure assessment scenario*). These scenarios are discussed in detail below.

These scenarios do not consider the consumption of food supplements (FC 17.1, FC 17.2 and FC 17.3), which is covered in an additional exposure scenario (*food supplement consumers only scenario*).

A possible additional exposure from the use of modified starches E 1404, E 1410, E 1412, E 1413, E 1414, E 1420, E 1422, E 1440, E 1442, E 1450 and E 1451 as carriers in food additives at QS, as food additives (including carriers) in food enzymes and in all nutrients at QS, in accordance with Annex III to Regulation (EC) No 1333/2008 (Parts 1, 3 and 5) was not considered in any of the exposure assessment scenarios, due to the absence of information on use levels.

3.8.1.1. Maximum level exposure assessment scenario

The regulatory maximum level exposure assessment scenario is based on the MPLs as set in Annex II to Regulation (EC) No 1333/2008 and listed in Table 14. As modified starches are authorised at QS in almost all food categories, a *maximum level exposure assessment scenario* was estimated based on the maximum reported use levels provided by food industry (excluding exposure via food supplements and foods for special medical purposes (FSMP)) as described in the EFSA Conceptual framework (EFSA ANS Panel, 2014). The maximum reported use levels used in this exposure scenario are listed in Appendix C.

The Panel considered the exposure estimates derived following this scenario as the most conservative, as it is assumed that the population group will be exposed to these modified starches present in food at the maximum reported use levels over a longer period of time, and assuming that modified starches are only used in the food categories for which data were submitted by food industry.

3.8.1.2. Refined exposure assessment scenarios

The refined exposure assessment scenario is based on use levels reported by industry. This exposure scenario can consider only food categories for which the above data were available to the Panel.

Appendix C summarises the concentration levels of modified starches used in the refined exposure assessment scenarios. Based on the available dataset, the Panel calculated two refined exposure estimates using different model populations, and excluding exposure via food supplements and FSMP:

- The brand-loyal consumer scenario: it was assumed that a consumer is exposed long-term to modified starches present at the maximum reported use level for one food category. This exposure estimate is calculated as follows:
 - Combining food consumption with the maximum of the reported use levels for the main contributing food category at the individual level;
 - Using the mean of the typical reported use levels for the remaining food categories.
- The non-brand-loyal consumer scenario: it was assumed that a consumer is exposed long-term to modified starches present at the mean reported use levels in food. This exposure estimate is calculated using the mean of the typical reported use levels.

3.8.1.3. Specific exposure assessment scenarios

- *Food supplement consumers only* scenario: Modified starches are authorised in the food category 17 Food supplements as defined in Directive 2002/46/EC, excluding food supplements for infants and young children. As exposure via food supplements may deviate largely from the one via other food, and the number of food supplement consumers may be low depending on populations and surveys, an additional scenario was calculated in order to reflect the additional exposure to food additives from food supplements, compared to exposure to food additives excluding these sources. This scenario was estimated as follows:
 - Consumers only of food supplements were assumed to be exposed to modified starches present at the maximum reported use level on a daily basis via consumption of food supplements. For the remaining food categories, the mean of the typical reported use levels was used.

As food category 17 does not include food supplements for infants and toddlers (Regulation (EC) No 1333/2008), exposure to modified starches from food supplements was not estimated for these two population groups.

Separate food supplements exposure scenarios based on the MPLs and the maximum reported levels for consumers only were carried out for starch aluminium octenyl succinate (E 1452), which is only authorised in food supplements as defined in Directive 2002/46/EC due to its use in vitamin preparations for encapsulation purposes. Exposure to aluminium from starch aluminium octenyl succinate was also estimated.

- *FSMP consumers only* scenario:

As modified starches are also authorised in the food category 13.1.5 (13.1.5.1 and 13.1.5.2), an additional exposure assessment scenario taking into account this food category was performed to estimate the exposure of infants and toddlers who may eat and drink these FSMP:

- Consumers only of FSMP were assumed to be exposed to modified starches present at the maximum reported use level on a daily basis via consumption of food categories 13.1.5.1 and 13.1.5.2.
- For the remaining food categories, the mean of the typical reported use levels was used.

The consumption of these foods under FC 13.1.5 is not reported in the EFSA Comprehensive database. To consider the exposure to food additives via consumption of these foods, the Panel assumed that the amount of FSMP consumed by infants and toddlers resembles that of comparable foods by infants and toddlers from the general population. Thus, the consumption of FSMP categorised as food category 13.1.5 is assumed to equal that of formulae and food products categorised as food categories 13.1.1, 13.1.2, 13.1.3 and 13.1.4.

FSMP consumed in other population groups (children, adolescents, adults and the elderly) may be very diverse; they cannot be considered because of very limited information on consumption. Eating

occasions belonging to the food categories 13.2, 13.3 and 13.4 were therefore reclassified under food categories in accordance to their main component.

These scenarios do not consider the consumption of food supplements.

3.8.1.4. Dietary exposure to modified starches (E 1404–E 1451)

Table 17 summarises the estimated exposure to modified starches from their use as food additives in six population groups (Table 16) according to the different exposure scenarios (Section 3.8.1). Detailed results per population group and survey are presented in Appendix D.

Table 17: Summary of dietary exposure to modified starches (E 1404–E 1451) from their use as food additives in the maximum level exposure assessment scenario and in the refined exposure assessment scenarios, in six population groups (minimum–maximum across the dietary surveys in mg/kg bw per day)

	Infants (12 weeks– 11 months)		Toddlers (12–35 months)		Children (3–9 years)		Adolescents (10–17 years)		Adults (18–64 years)		The elderly (≥ 65 years)	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Maximum level exposure assessment scenario												
Mean	36	1,527	296	2,087	424	1,766	212	1,093	142	591	113	474
95th percentile	140	4,219	849	3,670	854	3,386	462	2,188	418	1,382	335	1,118
Refined estimated exposure assessment scenarios												
Brand-loyal scenario												
Mean	29	797	247	1,455	312	1,332	149	760	104	468	88	415
95th percentile	109	2,567	735	3,053	667	2,998	309	1,601	285	1,202	240	1,020
Non-brand-loyal scenario												
Mean	22	505	141	790	193	652	110	406	55	218	40	170
95th percentile	84	1,947	402	1,448	419	1,160	241	777	155	453	104	349

In the *maximum level exposure assessment scenario*, mean exposure to modified starches from their use as food additives ranged from 36 mg/kg bw per day in infants to 2,087 mg/kg bw per day in toddlers. The 95th percentile of exposure to modified starches ranged from 140 to 4,219 mg/kg bw per day in infants.

In the *refined estimated exposure assessment scenario*, in the *brand-loyal scenario*, mean exposure to modified starches from their use as food additives ranged from 29 mg/kg bw per day in infants to 1,455 mg/kg bw per day in toddlers. The 95th percentile of exposure to modified starches ranged from 109 mg/kg bw per day in infants to 3,053 mg/kg bw per day in toddlers. In the *non-brand-loyal scenario*, mean exposure to modified starches from their use as food additives ranged from 22 mg/kg bw per day in infants to 790 mg/kg bw per day in toddlers. The 95th percentile of exposure to modified starches ranged from 84 to 1,947 mg/kg bw per day in infants.

In the *maximum level exposure assessment scenario* and in the *refined brand-loyal exposure assessment scenario*, the main contributing food categories to the total mean exposure estimates were: foods for infants and young children, fine bakery wares, unflavoured fermented milk products for infants; flavoured fermented milk products, snacks and flavoured drinks for toddlers and children; fine bakery wares, flavoured drinks and snacks for adolescents and adults; and fine bakery wares, sauces and soups for the elderly. In the *non-brand-loyal scenario*, the main contributing food categories were almost the same, except that for infants, flavoured fermented milk products, and for the elderly, fat and oil emulsions, mainly of water-in-oil type (e.g. margarines), were also considered important contributing categories. The main food categories contributing to the exposure to modified starches are presented in Appendix E.

In the *food supplement consumers only exposure scenario*, mean exposure to modified starches (E 1404–E 1451) from their use as food additives ranged from 220 to 876 mg/kg bw per day for children and from 108 to 210 mg/kg bw per day for adults. The 95th percentile of exposure ranged from 439 to 991 mg/kg bw per day for children and from 264 to 434 mg/kg bw per day for adults.

In the *FSMP consumers only scenario* for infants and toddlers, the mean exposure ranged from 362 mg/kg bw per day for toddlers to 3,466 mg/kg bw per day for infants. For the same age groups, the 95th percentile exposure ranged from 1,085 mg/kg bw per day for toddlers to 5,286 mg/kg bw per day for infants.

3.8.1.5. Dietary exposure to starch aluminium octenyl succinate (E 1452)

Table 18 summarises the estimated exposure to starch aluminium octenyl succinate (E 1452), from its use as a food additive in food supplements in four population groups (Table 16) according to the specific exposure assessment scenarios for food supplement consumers only (Section 3.8.1.3).

Table 18: Summary of dietary exposure to E 1452 from its use as a food additive in the regulatory maximum level (MPL) exposure assessment scenario and in the maximum reported level exposure assessment scenario, in four population groups (minimum–maximum across the dietary surveys in mg/kg bw per day)

	Children (3–9 years)		Adolescents (10–17 years)		Adults (18–64 years)		The elderly (≥ 65 years)	
	Min	Max	Min	Max	Min	Max	Min	Max
Regulatory maximum level (MPL) exposure assessment scenario - consumers only								
Mean	1.45	12.13	0.92	2.92	0.40	3.13	1.13	8.73
95th percentile	4.24	17.07	2.24	3.57	2.54	15.21	3.40	22.12
Maximum reported level exposure assessment scenario – consumers only								
Mean	0.08	0.65	0.05	0.16	0.02	0.17	0.06	0.47
95th percentile	0.23	0.91	0.12	0.19	0.14	0.81	0.18	1.18

In the *food supplement consumers only regulatory maximum level (MPL) exposure assessment* scenario, mean exposure to E 1452 from its use as a food additive in food supplements ranged from 0.4 mg/kg bw per day in adults to 12.1 mg/kg bw per day in children. The 95th percentile of exposure to E 1452 ranged from 2.2 mg/kg bw per day in adolescents to 22.1 mg/kg bw per day in the elderly.

In the *food supplement consumers only maximum reported level exposure assessment* scenario, mean exposure to E 1452 from its maximum reported use as a food additive in food supplements ranged from 0.02 mg/kg bw per day in adults to 0.65 mg/kg bw per day in children. The 95th percentile of exposure to E 1452 ranged from 0.12 mg/kg bw per day in adolescents to 1.2 mg/kg bw per day in the elderly.

3.8.1.6. Uncertainty analysis

Uncertainties in the exposure assessment of modified starches have been discussed above. In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2007a,b), the following sources of uncertainties have been considered and summarised in Table 19.

Modified starches are authorised as Group I food additives in 67 food categories and also have specific authorised uses in five other food categories (Table 14). Since the majority of food categories correspond to the general Group I food additives authorisation, modified starches may not necessarily be used in some of these food categories. This may explain why use levels of modified starches were not reported by the food industry for 36 food categories. However, the Panel noted that information from the Mintel GNPD (Appendix B) indicated that some of these 36 food categories were labelled with modified starches (e.g. processed fish and fishery products including molluscs and crustaceans, breakfast cereals).

Overall, the Panel considered that the uncertainties identified would, in general, result in an overestimation of the exposure to modified starches as food additives in European countries for the maximum level exposure scenario. The Panel noted that food categories which may contain modified starches due to carry-over (Annex III, Part 1, 3, 5) were not considered in the current exposure assessment.

Table 19: Qualitative evaluation of influence of uncertainties on the dietary exposure estimate for the general population

Sources of uncertainties	Direction ^(a)
Consumption data: different methodologies/representativeness/underreporting/misreporting/no portion size standard	+/-
Use of data from food consumption survey of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Correspondence of reported use levels to the food items in the EFSA Comprehensive Food Consumption Database: uncertainties to which types of food the levels refer to	+/-
Uncertainty in possible national differences in use levels of food categories	+/-
Food categories selected for the exposure assessment: exclusion of food categories due to missing FoodEx linkage (n = 10 out of 72 food categories)	-
Food categories selected for the exposure assessment: inclusion of food categories without considering the restrictions/exceptions (n = 4 out of 72 food categories)	+
Food categories included in the exposure assessment: data not available for certain food categories, which were excluded from the exposure estimates (n = 36 only for the refined scenarios out of 72 food categories)	-
12–79% of the amount of food consumed taken into account in the refined exposure assessment scenarios out of all authorised food (n = 36 out of 72 food categories)	
<i>Maximum level exposure assessment scenario (E 1404–E 1451):</i>	
exposure calculations based on the maximum reported use levels (reported from food industry)	+
food categories which may contain modified starches due to carry-over from uses according to Annex III to Regulation (EC) No 1333/2008 not considered	-
assumption that the food additive is not used in the food categories in which it is authorised at QS and for which no use levels were submitted	-
<i>Refined exposure assessment scenarios (E 1404–E 1451):</i>	
exposure calculations based on the maximum or mean use levels (reported from food industry)	-
food categories which may contain modified starches due to carry-over according to Annex III to Regulation (EC) No 1333/2008 not considered	+/-
assumption that the food additive is not used in the food categories for which no use levels were submitted	-
<i>Food supplement consumers only scenario (E 1404–E 1451):</i>	
exposure calculations based on consumers only	+
exposure calculations based on the mean use levels (reported from food industry) for all foods, except food supplements	-
foods which may contain the food additive according to Annex III to Regulation (EC) No 1333/2008 not taken into account	-
<i>Food supplement consumers only scenario for E 1452/aluminium:</i>	
exposure calculations based on consumers only	+
the restriction in the permitted use (only in vitamin preparations for encapsulation purposes) cannot be taken into account	+
<i>FSMP consumers only scenarios:</i>	
exposure calculations based on consumers only	+
exposure calculations based on the maximum levels for FSMP and mean levels for all other foods	+/-
foods which may contain the food additive according to Annex III to Regulation (EC) No 1333/2008 not taken into account	-

(a): +, uncertainty with potential to cause overestimation of exposure; -, uncertainty with potential to cause underestimation of exposure.

For the exposure assessment scenario for starch aluminium octenyl succinate (E 1452), taking into account the entire food category of food supplements (FC 17.1, 17.2 and 17.3), the Panel considered that the uncertainties identified would result in an overestimation of the exposure, as E 1452 is authorised as a food additive and carrier in food supplements as defined in Directive 2002/46/EC, due to its use in vitamin preparations for encapsulation purposes only.

3.8.2. Exposure to aluminium from the use of E 1452

According to the EU purity criteria, the content of aluminium in starch aluminium octenyl succinate (E 1452) is limited to a maximum of 0.3%. Based on this information, exposure to aluminium from the use of E 1452 as a food additive in food supplements for the *regulatory maximum level exposure assessment* scenario and the *maximum reported level exposure assessment* scenario was also assessed.

Table 20 summarises the estimated exposure to aluminium from the use of starch aluminium octenyl succinate (E 1452) as a food additive in food supplements in four population groups (Table 16) according to the different exposure scenarios (Section 3.8.1.5).

Table 20: Summary of dietary exposure to aluminium from the use of starch aluminium octenyl succinate (E 1452) as a food additive in food supplements at the regulatory maximum level (MPL) exposure assessment scenario and in the maximum reported level exposure assessment scenario, in four population groups (minimum–maximum across the dietary surveys in mg/kg bw per day)

	Children (3–9 years)		Adolescents (10–17 years)		Adults (18–64 years)		The elderly (≥ 65 years)	
	Min	Max	Min	Max	Min	Max	Min	Max
Regulatory maximum level exposure assessment scenario								
Mean	0.0044	0.0364	0.0028	0.0088	0.0012	0.0094	0.0034	0.0262
95th percentile	0.0127	0.0512	0.0067	0.0107	0.0076	0.0456	0.0102	0.0664
Maximum reported level exposure assessment scenario								
Mean	0.00023	0.00195	0.00015	0.00047	0.00006	0.00050	0.00018	0.00140
95th percentile	0.00068	0.00274	0.00036	0.00057	0.00041	0.00244	0.00055	0.00355

Mean exposure to aluminium from the use of E 1452 as a food additive in the *maximum regulatory level exposure assessment* scenario ranged from 0.0012 mg/kg bw per day in adults to 0.0364 mg/kg bw per day in children. The 95th percentile of exposure to aluminium ranged from 0.0067 mg/kg bw per day in adolescents to 0.0664 mg/kg bw per day in the elderly.

Mean exposure to aluminium from the use of E 1452 as a food additive in the *maximum reported level exposure assessment* scenario ranged from 0.00006 mg/kg bw per day in adults to 0.00195 mg/kg bw per day in children. The 95th percentile of exposure to aluminium ranged from 0.00036 mg/kg bw per day in adolescents to 0.00355 mg/kg bw per day in the elderly.

Furthermore, according to the information provided by industry, the content of aluminium in E 1452 is significantly lower (0.0004–0.16%) than the limit set in the EU specifications for E 1452.

3.8.3. Exposure via other sources

This re-evaluation refers exclusively to the use of modified starches as food additives in food, including food supplements, and does not include a safety assessment of other uses of modified starches.

Pharmaceutical uses

From data provided by the European Medicines Agency (EMA), information about the current medicinal usage of modified starches and their usage as an excipient, was retrieved (Documentation provided to EFSA n. 11).

Modified starches are used as excipients in medicinal products and, in particular, in medicinal products that come in various types of tablet forms, such as sustained/extended release tablets,

film-coated tablets, orally disintegrating tablets and chewable tablets (mostly analgesics). Their function in pharmaceuticals is described as tablet disintegrant for immediate drug release, as controlled/sustained release polymer for drugs and hormones (Singh and Nath, 2010; Ochubiojo and Rodrigues, 2012; Rumman et al., 2015) and for encapsulation purposes. They are also used in medicinal products that come in powder form (such as antiflu preparations to be taken orally or antifungal powders for local external use) and as plasma volume expander for trauma, heavy blood loss and cancer.

Commonly used modified starches are oxidised starch, acetylated starch, hydroxypropyl starch, hydroxypropyl distarch phosphate and starch phosphate. Furthermore, starch aluminium octenyl succinate (E 1452) is extensively used in corticosteroid medicinal creams to be applied dermally for the treatment of various skin diseases such as eczema.

4. Biological and toxicological data

Toxicity data were not available for all of the modified starches evaluated in the present opinion and for all endpoints. In general, the most complete datasets were available for acetylated distarch phosphate (E 1414) and acetylated distarch adipate (E 1422). However, given their structural, physicochemical and biological similarities, the Panel considered it possible to read-across between all the modified starches.

4.1. Absorption, distribution, metabolism and excretion

There is evidence that certain high molecular weight dietary polysaccharides, such as starches, could be partially broken down by enzymes (e.g. amylase) in the digestive tract of man. Thereafter, intermediate metabolites, such as lactic, acrylic or fumaric acid, are formed and the main end products of the colonic anaerobic digestive process by bacteria are short-chain fatty acids (SCFA) such as acetic, propionic and butyric acids, which are absorbed from the colon (Cummings and Englyst, 1987).

The following *in vitro* data on microbial fermentation of the two major components of starches, amylose and amylopectin, were available:

A total of 188 strains of 10 species of *Bacteroides* found in the human colon were tested for their ability to ferment mucins and plant polysaccharides (Salysers et al., 1977a). Many of the *Bacteroides* strains tested were able to ferment a variety of plant polysaccharides, including amylose, dextran, pectins and gums. The ability to utilise mucins and plant polysaccharides varied considerably among the *Bacteroides* species. Amylose and amylopectin were shown to be mainly fermented by 8 species of *Bacteroides*.

A total of 154 strains of 22 species of *Bifidobacterium*, *Peptostreptococcus*, *Lactobacillus*, *Ruminococcus*, *Coprococcus*, *Eubacterium* and *Fusobacterium*, which are present in high concentrations in the human colon, were tested for their ability to ferment 21 different complex carbohydrates (Salysers et al., 1977b). Among them, amylose and amylopectin were fermented by many strains of *Bifidobacterium*, *Eubacterium*, *Peptostreptococcus* and *Ruminococcus* species.

Two hundred and ninety strains of 29 species of bifidobacteria of human and animal origin (mainly of faecal origin) were tested for their ability to ferment complex carbohydrates (Crociani et al., 1994). Amylose and amylopectin were among the substrates fermented by the largest number of species (22 out of 29 species tested).

Fermentation of 10 polysaccharides, including amylopectin, by species of the family Enterobacteriaceae (Klebsiellae and other gram-negative facultative bacillia) was examined by Ochuba and Von Riesen (1980). Amylopectin was fermented by most of the species, including *Klebsiella*, *Enterobacter*, *Aeromonas*, *E. coli*, *Vibrio* and *Yersinia* strains. According to the authors, this study demonstrated the fermentation of amylopectin by enteric bacteria.

4.1.1. Oxidised starch (E 1404)

In vitro study

In vitro digestibility by pancreatin or saliva was used to compare slightly and highly oxidised corn starch with unmodified cornstarch and reference starch (unspecified compounds). Maltose production following a fixed interval of enzyme action was taken as a measure of digestibility. The oxidised starch was 10–15% less digestible by pancreatin than unmodified starch, but there was no obvious difference as regards salivary digestion (Documentation provided to EFSA n. 12).

***In vivo* studies**

The digestibility²⁰ of a great number of unmodified or modified starches was compared in rats (Booher et al., 1951). Among them, the digestibility of a commercial wheat preparation oxidised with hypochlorite, a 'thin-boiling' product, was tested in weanling male rats (n = 6, body weight 45–60 g, strain not specified) over a feeding period of 28 days. Matched-feeding techniques were used, with the modified starch as the sole source of carbohydrate at a level of 63.7% (on dry basis) of the diet. Body weight changes, faecal residues and digestibility coefficients for each starch were investigated. After sacrifice, changes were noted in the gastrointestinal tracts of the animals. The digestibility coefficients were calculated from the starch content of ingested food, residues found in faeces and post-mortem gastrointestinal contents. There were no significant differences in body weight gain and digestibility coefficients between the oxidised and the corresponding control wheat starch. Post-mortem examination showed no differences.

Cornstarch oxidised with 2.5%, 6% and 43.2% hypochlorite calculated as chlorine (= carboxyl groups introduced: 0.32%; 1.15 COOH per 100 glucopyranose units, 0.9%; 3.81 COOH per 100 glucopyranose units, 1.46%; 5.23 COOH per 100 glucopyranose units) was fed to rats (6 animals per sex and group, Wistar–Purdue strain) (Whistler and Belfort, 1961). The animals were kept for 7 days on 5 g basal diet and then given either 1 or 2 g starch supplements for 21 days. The weight gain was unaffected. Poor weight gain with diarrhoea was noted only with the highly oxidised material (43.2%) at both dietary levels. One rat from each of the high dietary level groups was examined. Marked caecal dilation was seen only in animals fed the highly oxidised starch. According to the authors, this very highly oxidised non-commercial starch was prepared only to determine whether any effect on weight gain could be produced by feeding an overoxidised product.

Cornstarch oxidised with 3.9%, 4.5% or 5.5% hypochlorite calculated as chlorine (= carboxyl groups introduced: 0.57%; 2.04 COOH groups per 100 glucopyranose units, 0.8%; 2.86 COOH groups per 100 glucopyranose units, 0.9%; 3.57 COOH groups per 100 glucopyranose units) was fed to rats (3 animals per group, strain and sex not specified) for 10 days (White, 1963). One, 2 or 4 g modified or control starch were added to 5 g basal diet. With increasing oxidation, digestibility slightly decreased, but no effect on caloric values was observed. No tissue damage was associated with the diarrhoea and caecal enlargements observed in the groups receiving 2 or 4 g starch. Liver, kidney, heart and spleen weights were normal. Diarrhoea and caecal enlargement are known to occur in rats fed starches of poor digestibility or other carbohydrates.

4.1.2. Monostarch phosphate (E 1410)

***In vitro* studies**

In vitro enzymatic hydrolysis of monostarch phosphate by wheat α -amylase was compared with that of unmodified wheat starch. No difference was noted in the rate of production of reducing substances (LIMCC, 1955; cited in JECFA, 1974a).

***In vivo* studies**

The nutritional value of chemically modified cornstarches was compared in male and female rats (6 animals per sex and group, Wistar–Purdue strain) (Whistler and Belfort, 1961). Animals were fed daily 5 g of a balanced diet supplemented with 1 or 2 g of cornstarch or modified starches, including corn starch phosphate (0.5–0.9 DS), for 21 days. Cornstarch phosphate produced similar weight gain as the commercial unmodified cornstarch, used as control.

The metabolic behaviour of the phosphate radical in starch phosphate was studied *in vivo* by comparing the distribution of ³²P after oral administration of labelled starch phosphate to rats, with that from either labelled orthophosphate or pyrophosphate (LIMCC, 1955; cited in JECFA, 1974a). The percentage activity excreted in urine and faeces over 48 h, as well as the percentage activity retained in liver, kidney, blood plasma and bone, showed no significant difference for the three types of phosphate examined. The phosphate moiety of starch phosphate probably behaves metabolically like any other ionic phosphate. The Panel considered that the complete *in vivo* dissociation of the phosphate radicals would give evidence of the complete degradation of starch phosphates in the animal body.

²⁰ Digestibility of a food component is measured as the difference between the intake and the faecal output of the unchanged component (from 'A dictionary of food and nutrition', 2005).

4.1.3. Distarch phosphate (E 1412)

***In vitro* studies**

The *in vitro* digestibility of a distarch phosphate using trimetaphosphate, by salivary, pancreatic and intestinal amylase was measured by the production rate of reducing sugar (Rosner, 1960; cited in JECFA, 1974b). No deleterious effect was shown on enzymic depolymerisation.

The *in vitro* digestibility of distarch phosphate (E 1412) and phosphated distarch phosphate (E 1413) by pancreatic amylase were compared (Documentation provided to EFSA n. 13). Following an incubation of 20 min, the digestibility of the modified starches measured by the production of maltose was similar or slightly reduced (by 18%) to that of normal starches.

The *in vitro* digestibility, by pancreatin, of corn or potato starch modified with 0.05% or 0.1% phosphorus oxychloride was found to be similar to that of the unmodified starch (Janzen, 1969; unpublished report, cited in JECFA, 1974b). When starch modified with 0.5% or 1.5% of phosphorus oxychloride was used, the resulting cross-linkage considerably inhibited digestibility *in vitro* in a manner related to the concentration of cross-linking agent used.

Distarch phosphate (Fielders Pty Ltd., Australia, DS 0.06) was hydrolysed with porcine pancreatic α -amylase (~ 0.4 mg) for 1 h (Wootton and Chaudhry, 1979). The digestibility was estimated by increase in reducing power and by decrease in the ratio of spectrophotometric absorbance of digested to undigested starches (blue value). The digestibility of distarch phosphate after 60 min was $79.3 \pm 2.0\%$ and similar to that of unmodified wheat starch used as a control ($81.3 \pm 0.6\%$).

Modified potato starches, including distarch phosphate (in a raw, as well as in a drum-dried form, Starkelsen, Sweden) were hydrolysed with porcine pancreatic α -amylase (~ 1 mg) for 1 h (Oestergaard et al., 1988). The rate of hydrolysis of distarch phosphate with pancreatic α -amylase after 60 min was 95–100% compared to that of unmodified starch. Raw products, not boiled prior to hydrolysis, showed similar results as drum-dried products. According to the authors, cross-linking with phosphate only did not appear to reduce the rate of hydrolysis with α -amylase.

***In vivo* studies**

The caloric value and digestibility of a distarch phosphate using trimetaphosphate were tested in groups of 10 rats fed for 7 days a 4 g basal diet supplemented with either 0.9 or 3.6 g starch (Hixson, 1960; cited in JECFA, 1974b). No significant differences were observed in body weight gain and liver, kidney, heart and spleen weights after the feeding period between animals fed the modified and the unmodified starches.

The *in vivo* digestibility was examined in groups of 10 male rats fed for 10 days with a 5 g basal diet supplemented with 1, 2 or 4 g trimetaphosphate modified starch or two 'reference starches' used as controls (Documentation provided to EFSA n. 14). Weight gains were identical for all types of starch tested at all three levels of supplementation. No unusual behavioural reactions were observed.

The caloric value of starch treated with 0.06% phosphorus oxychloride was determined in groups of six male and six female rats receiving a diet containing 52% distarch phosphate as the sole carbohydrate source, for 6 weeks (Oser, 1954; cited in JECFA, 1974b). No differences were noted between rats fed modified and unmodified starches.

4.1.4. Phosphated distarch phosphate (E 1413)

***In vitro* studies**

The *in vitro* digestibility of phosphated distarch phosphate (E 1413) (prepared from cornstarch), by pancreatic amylase, was somewhat reduced (measured as maltose, 118 mg maltose), compared with 'reference starches' (141 mg maltose) after 20 min (Documentation provided to EFSA n. 13).

The *in vitro* digestibility of two maize starches (unmodified and phosphated distarch phosphate) was compared by using incubation (5 h) with pancreatin, in combination with porcine mucosal enzymes (Documentation provided to EFSA n. 15). The phosphated modified starch (up to 0.04% phosphorus) showed a similar *in vitro* digestibility (95%, no information on statistical significance) when compared to the unmodified maize starch.

***In vivo* studies**

The *in vivo* digestibility was examined in groups of 10 male rats fed for 10 days with a 5 g basal diet supplemented with 1, 2 or 4 g unmodified starch, distarch phosphate (E 1412) or phosphated

distarch phosphate (E 1413) (Documentation provided to EFSA n. 14). Weight gains were comparable for all types of starches tested at all three levels of supplementation. No unusual behavioural reactions were observed.

4.1.5. Acetylated distarch phosphate (E 1414)

***In vitro* studies**

The digestibility of various potato starches, including unmodified potato starch and acetylated distarch phosphate (modified to 2.3% acetyl content) was compared by using incubation (5 h) with pancreatin, in combination with porcine mucosal enzymes (Documentation provided to EFSA n. 15). The acetylated distarch phosphate showed a lower *in vitro* digestibility (81%, no information on statistical significance) when compared to the unmodified potato starch used as control.

Acetylated distarch phosphate (Fielders Pty Ltd., Australia, DS 0.04) was hydrolysed with porcine pancreatic α -amylase (about 0.4 mg) for 1 h (Wootton and Chaudhry, 1979). The digestibility was estimated by increase in reducing power and by decrease in the ratio of spectrophotometric absorbance of digested to undigested starches (blue value). The digestibility of acetylated distarch phosphate after 60 min was $75.2 \pm 0.2\%$, compared to $81.3 \pm 0.6\%$ for the unmodified wheat starch, used as control.

Modified potato starches, including acetylated distarch phosphate (in a raw, as well as in a drum-dried form, Starkelsen, Sweden), were hydrolysed with porcine pancreatic α -amylase (about 1 mg) for 1 h (Oestergaard et al., 1988). The rate of hydrolysis of acetylated distarch phosphate with pancreatic α -amylase after 60 min was 71–97% compared to that of unmodified starch. Raw products, not boiled prior to hydrolysis, showed similar result as drum-dried products.

4.1.6. Acetylated starch (E 1420)

***In vitro* studies**

Digestibility of acetylated starches (not further specified) by fungal amyloglucosidase was shown to be 68–81% of that of native starch (Kruger, 1970; unpublished report, cited in JECFA, 1982f).

The digestibility of various potato starches, including unmodified starch and starch acetate (modified to 1.98% acetyl content) was compared by using incubation (5 h) with pancreatin, in combination with porcine mucosal enzymes (Documentation provided to EFSA n. 15). The starch acetate showed a lower *in vitro* digestibility (90%, no information on statistical significance) when compared to the unmodified potato starch used as a control.

Acetylated starch (Fielders Pty Ltd., Australia, DS 0.07) was hydrolysed with porcine pancreatic α -amylase (about 0.4 mg) for 1 h (Wootton and Chaudhry, 1979). The digestibility was estimated by increase in reducing power and by decrease in the ratio of spectrophotometric absorbance of digested to undigested starches (blue value). The digestibility of acetylated starch after 60 min was $70.5 \pm 0.8\%$ compared to $81.3 \pm 0.6\%$ for unmodified wheat starch used as control.

***In vivo* studies**

Caloric value was determined in groups of 10 male rats fed for 4 weeks a diet supplemented with graded doses of 0, 1.5, 3.0, 4.5 and 6.0 g dextrose (equivalent to 0, 6, 12, 18 and 24 calories) (Oser, 1961a; cited in JECFA, 1982f). The dose–response curve was used to estimate the caloric value of supplements of 3 and 4.5 g of acetylated (1.8% acetyl) or native starch. No significant difference was found between the starch samples with regard to caloric value.

Preliminary to *in vivo* studies, the digestibility of an acetylated starch containing 2.5% acetyl groups ('type 2.5–0.20') was measured by the biochemical oxygen demand (BOD) of samples incubated with bacteria or fungal amyloglucosidase ((Documentation provided to EFSA n. 16). An increase of the acetyl content corresponded to decreased BOD values and decreased digestibility. Starch acetate was only 93.7% as digestible as native starch. In a first *in vivo* experiment, the effect of varying the degrees of acetylation and cross-linking of native starch on the weight gain, feed efficiency, caecal weight and incidence of diarrhoea was measured in male weanling rats during 28 days of feeding. Acetylated starch containing 2.6% acetyl groups had no significant effect on feed efficiency or rate of survival, but slightly reduced the rate of weight gain. The observed caecal enlargement and diarrhoea were not accompanied by tissue damage or inflammation. In another *in vivo* experiment in rats, the relative digestibilities and caloric values of various acetylated starches (0.46, 1.77, 2.20 and 3.50 acetyl groups) were compared to those of native starch. The results of this experiment indicated that the

relative digestibilities and caloric values of native and acetylated starches were essentially equivalent under the conditions used in the experiment. The author concluded that 'acetylated starch type 2.5–0.20, which represents the highest degree of acetylation and cross-linking requested in this application, can be used safely as a complete replacement for native starch in human foods. Although it appears to digest somewhat more slowly than native starch, their relative digestibilities and caloric values are essentially equivalent. It causes no observable tissue damage or irritation.'

Annisson et al. (2003) described experiments in rats to determine whether feeding of starches acylated with acetate, propionate or butyrate could elevate SCFA concentrations in the large bowel. In this study, maize starch was acylated with acetic anhydride to produce the corresponding acetylated starch (DS of ~ 0.18). Body weight gain did not differ between rats fed acetylated starch or a control starch for 14 days. Large bowel pH was significantly lower and digesta mass significantly higher throughout the large bowel in rats fed the acylated starches. Caecal and distal colonic starch averaged 12 mg in rats fed the control starch and 103 mg in rats fed acetylated starch, respectively. Large bowel SCFA concentrations and pools were significantly higher in rats fed acetylated starch. In the caecum, acetate, propionate and butyrate pools were 280% higher in rats fed the acetylated starch than in those fed the control diet. In the distal colon, the corresponding increase was 320%. These data indicated that acetylated starches are resistant starches (RS) and raise large bowel SCFA concentrations, apparently through bacterial release of the esterified fatty acid and fermentation of the residual starch.

Human studies

The excretion of starch and esterified SCFA by ileostomy subjects after ingestion of acylated starches was described by Clarke et al. (2007). The aim of the study was to determine whether cooked, highly acylated starches were resistant to small intestine digestion in ileostomy volunteers. Volunteers consumed single doses of custards containing 20 g cooked acetylated, propionylated or butyrylated high-amylose maize starches (HAMSA, HAMSP and HAMSB, respectively) on each collection day. The amounts of starch and of esterified SCFA ingested and subsequently excreted in the stoma effluent were measured. Custards containing unacylated high-amylose maize starch (Hylon VII, HAMS) and low-amylose maize starch (3401C, LAMS) were consumed as controls. 73.9% of the esterified SCFA survived small intestine digestion, which showed the potential of acetylated starches to deliver specific SCFA to the large bowel. The resistance of starches to small intestine digestion, as measured by ileal excretion, was significantly greater for HAMSA, HAMSP, HAMSB and HAMS than for LAMS. The concentration of acetate in stoma digesta was higher than expected in all groups; this additional acid may have been derived from endogenous sources. The authors concluded that 'acylated starches are a potentially effective method of delivering significant quantities of specific SCFA to the colon in humans.'

4.1.7. Acetylated distarch adipate (E 1422)

In vitro studies

The *in vitro* digestibility of acetylated distarch adipate (code 78-1087; 2% acetate and 0.14% adipate) by pancreatin, compared to unmodified starch (control), was investigated (Documentation provided to EFSA n. 17). Two types of ^{14}C -labelled acetylated distarch adipates were used: (a) 1- ^{14}C -acetate, ^{12}C -adipate; and (b) ^{12}C acetate, 1,6- ^{14}C -adipate. The control starch was more extensively hydrolysed than the acetylated distarch adipate. Pancreatin, *in vitro*, failed to hydrolyse acetylated distarch adipate completely to yield free adipic acid and glucose, although the acetate ester bond was split to liberate free acetic acid from the same sample preparation.

The digestibility of acetylated distarch adipate *in vitro* using amyloglucosidase was 98.3% (Kruger, 1970; cited in JECFA, 1982f).

In vivo studies

The metabolic fate of adipate-esterified starch was investigated in male rats (strain not specified, body weight ~ 200 g) using 1,6- ^{14}C adipic acid (Documentation provided to EFSA n. 18). The rate of appearance of $^{14}\text{CO}_2$ was compared for 24 h, between starch esterified with 1,6- ^{14}C adipic acid and free 1,6- ^{14}C adipic acid in rats receiving 250 mg of starch by gavage. When free labelled adipic acid was mixed with base starch (unmodified), it was rapidly absorbed and completely metabolised and excreted by the rat. 99.3% of the ^{14}C -activity of the free adipic acid was recovered in the exhaled air, whereas 5.8% appeared in the urine; none was detected in the faeces, in the gastrointestinal tract or in the carcass. When labelled adipate was esterified to starch, it was not as readily available for

absorption or metabolism. However, it was metabolised by the rat, with the carbon of the adipate moiety entering the carbon pool and appearing in the exhaled air and urine. Only 70.5% of the ^{14}C -activity of the esterified adipic acid appeared in the respired air and 7.2% in the urine, whereas 24.5% was found in the faeces. Only traces of radioactivity appeared in the carcass.

The caloric equivalent of a modified starch, treated with 0.2% adipic anhydride and 5.5% acetic anhydride, was determined in groups of 10 male rats fed for 28 days on a basal diet containing either 1.5 or 3.0 g of starch supplement (Oser, 1961b; cited in JECFA, 1982b). Native starch was used as the control. Caloric values were determined from a dose-response curve obtained by the use of 0, 0.75, 1.5, 3.0 and 4.5 g of sucrose supplements (equivalent to 0, 3, 6, 12 and 18 calories per day). There was no difference in the caloric value between the modified and unmodified starches.

4.1.8. Hydroxypropyl starch (E 1440)

***In vitro* studies**

The *in vitro* digestibility, by pancreatin, of low (1 in 10) and high (4 in 10) substituted starches was estimated by comparing the amount of reducing material liberated with that formed from native wheat starch. No significant difference could be detected between low (1 in 10) and high (4 in 10) substituted starches compared with unmodified starch (Kay and Calandra, 1962; cited in JECFA, 1982d).

The *in vitro* digestibility of hydroxypropyl starches with various degrees of substitution was determined by incubation with a relatively large amount of pancreatin (4 mg) for 4 h (Leegwater and Luten, 1971). The digestibility was estimated from the reduction of an alkaline ferricyanide solution by the digests. It was found that the digestibility decreased exponentially with increasing DS. At DS of 0.04, 0.068, 0.135, 0.23 or 0.45, the digestibility was respectively 80%, 68%, 46%, 20% and 3.8% of that of unmodified starch used as control.

Hydroxypropyl starch (Fielders Pty Ltd, Australia, DS 0.06) was hydrolysed with porcine pancreatic α -amylase (about 0.4 mg) for 1 h (Wootton and Chaudhry, 1979). The digestibility was estimated by increase in reducing power and by decrease in the ratio of spectrophotometric absorbance of digested to undigested starches (blue value). The digestibility of hydroxypropyl starch after 60 min was $62.3 \pm 1.0\%$ compared to $81.3 \pm 0.6\%$ for unmodified wheat starch, used as control.

***In vivo* studies**

Excretion of hydroxypropyl-2- ^{14}C starch (DS 0.12) was investigated in one male rat (strain not specified, weight 143 g) ((Documentation provided to EFSA n. 19). A quantity of 15 mg were given by gavage as 0.75 mL of a 2% (w/v) aqueous solution of gelatinised hydroxypropyl-2- ^{14}C starch. Over the next 50 h, more than 95% of the radioactivity was excreted in the faeces and 4% in the urine. The urinary activity was probably derived from propylene glycol in the test material. At least six labelled compounds were detected by thin layer chromatography after *in vitro* digestion of the labelled starch by pancreatin. At least four compounds were detected after digestion by pancreatin in combination with enzymes from porcine intestinal mucosa. The major compound in the pancreatin/mucosal enzyme digest was also detected in the contents of both the small and large intestine of the rat, 4 h after administration. The major metabolite mentioned above was isolated on a larger scale from an enzyme digest of an unlabelled hydroxypropyl starch, and it was tentatively identified as a mixture of hydroxypropyl maltoses.

In a further study (Leegwater and Speek, 1972), several hydroxypropyl oligoglucoses were detected in the faeces of rats on diets containing hydroxypropyl starches with DS of 0.025, 0.047 or 0.106. The major components were tentatively identified as hydroxypropyl maltose, dihydroxypropyl maltotriose, and dihydroxypropyl maltotetraose. The digestibility of the hydroxypropyl starches was found to decrease with increasing DS.

Leegwater et al. (1972) confirmed these data in rats fed with diets containing hydroxypropylated potato starches with a DS of up to 0.11. In these animals, the major metabolite isolated from the faeces was shown, by mass spectrometry (MS) and proton magnetic resonance (PMR) spectrometry of its peracetate, to be 4-O-(2-O-[(RS)-2-hydroxypropyl]- α -D-glucopyranosyl)-D-glucopyranose.

4.1.9. Hydroxypropyl distarch phosphate (E 1442)

***In vitro* studies**

Hydroxypropyl distarch phosphate (Fielders Pty Ltd., Australia, DS 0.06) was hydrolysed with porcine pancreatic α -amylase (about 0.4 mg) for 1 h (Wootton and Chaudhry, 1979). The digestibility

was estimated by increase in reducing power and by decrease in the ratio of spectrophotometric absorbance of digested to undigested starches (blue value). The digestibility of hydroxypropyl distarch phosphate after 60 min was $60.8 \pm 0.7\%$, compared to $81.3 \pm 0.6\%$ for unmodified wheat starch, used as control.

Modified potato starches, including hydroxypropyl distarch phosphate (in a raw, as well as in a drum-dried form, Starkelsen, Sweden) were hydrolysed with porcine pancreatic α -amylase (about 1 mg) for 1 h (Oestergaard et al., 1988). The rate of hydrolysis of hydroxypropyl distarch phosphate with pancreatic α -amylase after 60 min was 58–60% compared to that of unmodified starch. Raw products, not boiled prior to hydrolysis, showed similar result to drum-dried products.

Studies on the *in vitro* digestibility of hydroxypropyl distarch phosphate (from tapioca) with pancreatic or fungal amylase, showed that the extent of hydrolysis depends on gelatinisation conditions (time, temperature and pH) of the starch (Hood, 1973).

***In vivo* studies**

Digestibility of hydroxypropyl distarch phosphate was tested in groups of five rats fed for 7 days on a basal diet supplemented with 0, 1 and 3 g modified or control starch. No difference in weight gain was observed (Prier, 1961; cited in JECFA, 1975a).

4.1.10. Starch sodium octenyl succinate (E 1450)

***In vitro* study**

The *in vitro* digestibility of OSA-modified starch by porcine pancreatic and human salivary α -amylase, a fungal (*Aspergillus niger*) glucoamylase and a barley β -amylase was compared with that of the corresponding unmodified starch from which it was prepared (NSCC, 1984; cited in JECFA, 2015). The digestibility of OSA-modified starch, measured by the rate of production of reducing substances, ranged from 83% to 98% of that of its corresponding native starch. It was suggested that the slight differences in the rate of digestibility were likely due to those anhydroglucose units in the starch substituted with OSA (about 1 in 50) inhibiting the hydrolysis of the α 1-4 and α 1-6 bonds. The *in vitro* enzyme digestibility of OSA-modified starch was comparable to that reported for other modified food starches.

***In vivo* studies**

In a caloric utilisation study, groups of 10 male albino rats each, 20–22 days old, were fed either 2.74 g of a basal diet, or the basal diet supplemented with 1.5 or 3.0 g cornstarch or 1.5 or 3.0 g starch sodium octenyl succinate (OS), or with 0.75, 1.5, 3.0 or 4.5 g sucrose for a period of 4 weeks. No adverse effects were noted during the test period. The weight gains of the test group fed starch or substitutes were similar to those in the sucrose fed group. The caloric value of the substituted cornstarch (OS) was similar to cornstarch (Anonymous, 1960; cited in JECFA, 1982g).

Based on metabolism studies with ^{14}C -labelled OSA in male Sprague–Dawley rats (8–10 weeks) and female Beagle dogs (RLMD, 1990; cited in JECFA, 2015), the authors concluded that both the rat and the dog were able to metabolise the labelled OSA, but neither were able to metabolise OSA to carbon dioxide and water. Instead, OSA was metabolised to tricarboxylic acid or was excreted unchanged.

Two proprietary infant formulas intended for use in infants aged 0–12 months were used in a study designed to determine the urinary excretion levels of OSA and its metabolites following administration to rats by gavage (MJRC, 1992; cited in JECFA, 2015). Juvenile rats (four per group, sex and strain not specified) were randomised to receive a single dose of: (1) a 28% weight per volume (w/v) aqueous suspension of proprietary formula 1 (control), (2) a 28% (w/v) suspension of proprietary formula 1 to which OSA (0.72 mg/mL) was added or, (3) a 28% (w/v) suspension of proprietary formula 2 containing OSA-modified starch, with an OSA content of 0.42 mg/mL. The total dose of OSA equivalents was zero for the proprietary formula 1 control group, 120 $\mu\text{mol/kg}$ bw for the proprietary formula 1 plus OSA group and 69.1 $\mu\text{mol/kg}$ bw for the proprietary formula 2 group. All animals were healthy, and no changes in behaviour were observed following administration. The total urinary excretion of OSA and its metabolites was approximately $35 \pm 12\%$ and $19 \pm 2\%$ of the oral dose in the proprietary formula 1 plus OSA and proprietary formula 2 groups, respectively. Higher levels of OSA and its metabolites were detected in the urine of rats administered proprietary formula 1 plus OSA compared with those administered proprietary formula 2, corresponding to the greater amount of total OSA equivalents administered.

Human studies

The excretion of OSA and its related metabolites was analysed in 17 hospitalised infants and children (aged 2 months–6 years) fed one of three commercial hydrolysed protein formulas containing OSA-modified starch for an unspecified duration (Kelley, 1991). Random or 24-h urine samples were collected, and urinary metabolites were identified using gas chromatography–mass spectrometry (GC–MS). In addition, plasma samples were collected from five patients and analysed for free fatty acids and organic acids. The results indicated that between 10% and 25% of the OSA hydrolysed from ingested OSA-modified starch was absorbed and excreted in the urine. The average amount of OSA absorbed was estimated to be approximately 50–70 mg/kg bw. The principal compounds identified in the urine were OSA and at least nine metabolites that appeared to be produced from the oxidation of OSA by a combination of microsomal and mitochondrial or peroxisomal processes. The levels of OSA detected in the urine ranged from 121 to 1,353 mg/g creatinine, whereas urinary levels of OSA-related metabolites ranged from 73 to 2,168 mg/g creatinine. In the plasma, measurable concentrations of OSA (9.5–57.9 $\mu\text{mol/L}$) were detected, but no other related metabolites were detected at concentrations higher than 1 $\mu\text{g/mL}$. Based on the molecular weight and mass fragmentation of the nine identified metabolites associated with the excretion of OSA, the author proposed that OSA is metabolised in infants by a combination of ω -, ω -1 and β -oxidation steps, similar to valproic acid.

One hundred and seven female healthy term infants (aged 2–16 days), comprising 55 infants administered a milk-based formula containing OSA-modified starch (concentration not specified) and 52 administered a milk-based formula containing distarch phosphate modified tapioca starch (control), were fed for 120 days *ad libitum* (MJNR, 1994; cited in JECFA, 2015). Urine samples collected on day 90 were analysed for OSA and related metabolites. In the infants consuming OSA-modified starch, urinary OSA levels ranged from 0 to 1,398.6 $\mu\text{g/mg}$ creatinine (mean of 546.1 $\mu\text{g/mg}$ creatinine). The concentration of 1,2,9-non-4-enetricarboxylate, a metabolite of OSA, ranged from 0 to 865.5 $\mu\text{g/mg}$ creatinine (mean of 343.8 $\mu\text{g/mg}$ creatinine).

4.1.11. Acetylated oxidised starch (E 1451)

No data were available.

4.1.12. Starch aluminium octenyl succinate (E 1452)

No data were available.

4.1.13. Summary

Data on *in vitro* degradation of modified starches by digestive enzymes indicated that their digestibility was slightly reduced or showed no differences when compared to the corresponding unmodified starches.

In studies using porcine pancreatic α -amylase, the digestibility of the starch appeared to be reduced by substitution with hydroxypropyl and acetate groups, while cross-linking with phosphate had a smaller effect on its digestibility. Etherification with hydroxypropyl groups reduced the digestibility of the starch to a larger extent than esterification with acetate.

The action of amylase on modified starches would lead to the formation of glucose, maltose or oligosaccharides and/or their modified derivatives.

The Panel noted that in the case of starch sodium octenyl succinate, as demonstrated in infants, free OSA and its oxidative metabolites would be excreted in urine.

Despite the absence of ADME data for two of the modified starches (E 1451 and E 1452) and the availability of *in vivo* studies in humans for only two modified starches (E 1420 and E 1450), the Panel considered the ADME database sufficient to conclude that modified starches would not be absorbed intact but significantly hydrolysed by intestinal enzymes and then fermented by the intestinal microbiota in animals and humans.

In vivo data are in agreement with *in vitro* studies indicating that the two major components of starches, amylose and amylopectin, would be fermented during their passage through the large intestine by strains of bacteria found in the human colon. The main end products of this colonic anaerobic digestive process are SCFA such as acetic, propionic and butyric acids, which are absorbed from the colon. Based on the available knowledge on the role of SCFA as end products of the fermentation of dietary fibres by the anaerobic intestinal microbiota (Topping and Clifton, 2001; Den

Besten et al., 2013), the Panel considered that their potential formation as fermentation products from modified starches does not raise any safety concern.

4.2. Toxicological data

4.2.1. Acute oral toxicity

No data on the acute oral toxicity of oxidised starch (E 1404), monostarch phosphate (E 1410), phosphated distarch phosphate (E 1413), acetylated distarch phosphate (E 1414), acetylated starch (E 1420), acetylated distarch adipate (E 1422), hydroxypropyl starch (E 1440), hydroxypropyl starch phosphate (E 1442), starch sodium octenyl succinate (E 1450), acetylated oxidised starch (E 1451) and starch aluminium octenyl succinate (E 1452) were available.

4.2.1.1. Distarch phosphate (E 1412)

Two acute toxicity studies with distarch phosphate were conducted using mice, rats, guinea pigs, rabbits and cats (Hodge, 1954, 1956 cited in JECFA 1974b). These tests gave high LD₅₀ values of between 7 and 35 g/kg bw, depending on the species. Only small numbers of animals were used but no deaths occurred from the quantities administered. Livers and kidneys of guinea pigs, rabbits and cats showed no histopathological abnormalities related to the administration of the modified starch.

Overall, acute oral toxicity data for distarch phosphate were available for several species. All LD₅₀ values were above 7 g/kg bw.

4.2.2. Short-term and subchronic toxicity

4.2.2.1. Oxidised starch (E 1404)

Starch treated at a level of 0.375% with chlorine was fed to weanling albino rats at 70% of their diet (equal to 63,000 mg/kg bw per day) for 10 weeks, using cornstarch as the control (Garton & Sons Co. Ltd., 1967; cited in JECFA, 1975a). Feeding was either unrestricted or followed the paired-feeding technique. No toxic effects were noted. No details of this work, carried out in 1944–1945, were available.

An oxidised starch, obtained by treating cornstarch with 5.5% chlorine, using sodium hypochlorite (carboxyl content 0.90), was fed to groups of 15 male and 15 female weanling albino Wistar rats at dietary levels of 0.5%, 10% or 25% for 90 days (equal to 450, 9,000 and 22,500 mg/kg bw per day) (Documentation provided to EFSA n. 20). General condition, behaviour and survival were not adversely affected at any dietary level of the modified starch. Growth and food intake showed no distinct differences between the test groups and the controls in either sex. The water content of the faeces was comparable in all groups. Gross and microscopic pathological examination showed no treatment-related pathological changes. Diarrhoea was not observed, but the production of faeces dry matter at 25% of the oxidised starch-treated animals was increased compared to the controls. This phenomenon was accompanied by an increase in the weight of the caecum, both filled and empty, at the 25% level, in females only. However, no histological changes could be detected in this organ. Haematological indices, biochemical blood values and urine composition showed no treatment-related differences. From the results of this study it was concluded that feeding of the modified starch at levels up to 25% in the diet in rats did not induce any distinct adverse effects.

4.2.2.2. Monostarch phosphate (E 1410)

No data were available.

4.2.2.3. Distarch phosphate (E 1412)

Rats

Groups of 10 male and 10 female Wistar rats received 0%, 5%, 15% and 45% of two types of distarch phosphate (equivalent to 4,500, 13,500 and 40,500 mg/kg bw per day) in their diet for 90 days (Documentation provided to EFSA n. 21). One type was cross-linked to the extent of a normal food starch, while the other was excessively cross-linked by maintaining a high pH during treatment and using a relatively high concentration of POCl₃ (0.085% and 0.128% esterified phosphate, respectively). No abnormalities, compared with controls, were seen as regards general appearance, behaviour, mortality, food consumption, haematology, serum chemistry and urinalysis, which could be ascribed to the action of either of the test substances. No diarrhoea or increased caecal weights were

observed. Gross and histopathological examinations revealed no abnormalities attributable to the test substances. It was concluded that the two starches modified with phosphorus oxychloride did not induce any treatment-related changes when fed to rats at dietary levels up to 45% for three months.

Dogs

Groups of three male and three female Beagle dogs were given daily for 90 days gelatine capsules containing 50, 250 and 1,250 mg/kg bw distarch phosphate (Documentation provided to EFSA n. 22). No significant differences in body weight among the groups were reported. Food consumption was comparable for all groups. No untoward behavioural reactions were noted during the entire testing period. The results of haematology, clinical blood chemistry, urine analyses and liver function tests did not show significant abnormalities. Gross or histopathological findings showed no adverse effects in any of the animals. Organ weight data and organ-body weight ratios calculated from these data did not reveal any significant inter-group differences.

Pigs

Groups of eight Pitman–Moore miniature pigs were weaned at 3 days of age, and were fed formula diets containing 5.4% unmodified starch or 5.6% distarch phosphate (treatment with 0.08% phosphorus oxychloride) for 25 days (Anderson et al., 1973a). Body weight gain was comparable among the groups. At termination of the study, no differences due to treatment were observed in any of the chemical values for blood (haemoglobin) and serum (cholesterol, triglyceride, calcium, phosphorus, alkaline phosphatase, urea nitrogen, total protein, albumin and globulin). Relative organ weight, as well as carcass composition (water, fat, protein, ash, Ca, PO₄, Na, Mg) and liver composition (water, fat, protein and ash) were similar for test and control animals.

4.2.2.4. Phosphated distarch phosphate (E 1413)

Rats

Groups of 10 male and 10 female rats (strain unspecified) were fed a diet containing 1%, rising to 35%, of phosphated distarch phosphate (cross-linking obtained by trimetaphosphate) for a total of 60 days (equivalent to 9,000–31,500 mg/kg bw per day) (Kohn et al., 1964a cited in JECFA, 1974b). The mean body weight gain showed a consistent reduction throughout the study in female rats. Although four test and two control animals died during the study, these incidents were regarded as unrelated to the test substance. All animals behaved normally. Haematology and urinalysis results were within physiological ranges and comparable among the various groups. The liver weights of male rats were lower for the test group than for controls and the kidney weights were lower for both sexes, but these findings were not associated with any gross or histopathological changes. The Panel noted the high number of animals that died during the study, therefore the results of the study were of limited value for risk assessment.

Groups of 10 male and 10 female rats (CIVO colony, Wistar derived) received in their diet 0%, 25% and 50% of phosphated distarch phosphate (0.3% phosphorus; code Snow Flake 4832) for 8 weeks (equivalent to 22,500 and 45,000 mg/kg bw per day) (Documentation provided to EFSA n. 23). There were no detectable adverse effects on body weight. Faecal water content appeared to be higher in animals fed the 50% modified starch, but the results were too variable to allow for any definite conclusions. Production of faeces appeared to be unaffected when compared with controls. No diarrhoea occurred at any test level. Caecal weight in males was slightly increased, but showed no dose-response relationship. Caecum weights of the females were very similar to, or even slightly lower than those of the corresponding control.

Groups of 10 male and 10 female rats (albino, not further specified) were fed a diet containing 10%, rising to a diet containing 35%, of phosphated distarch phosphate (E 1413) for 60 days (equivalent to 9,000–31,500 mg/kg bw per day) (Documentation provided to EFSA n. 24). Female rats showed consistent reduced weight gain throughout the test. Although four test and two control animals died during the test, these incidents were not attributed to ingestion of the test starch. No abnormal behavioural reactions were noted during the investigation. Haematological examination and urinalysis were normal and comparable in the various groups. The absolute liver weights of male rats were lower for the test group than for controls and the absolute kidney weights were lower for both sexes, but these findings were not associated with any gross or histopathological changes. The Panel noted the high number of animals that died during the study, therefore the results of the study were of limited value for risk assessment.

Groups of 25 male and 25 female rats (albino, not further specified) were fed diets containing 1.0 and 5.0% phosphated distarch phosphate (E 1413) or unmodified starch for 90 days (equivalent to 900 and 4,500 mg/kg bw per day) (Documentation provided to EFSA n. 25). Eleven controls and six test animals died. All deaths were attributed to respiratory disease. Body weight gain and food consumption showed no differences between the groups. Organ weights and haematological examination on day 45 and 90 showed no differences between the two groups. Pooled urinalysis was comparable for all groups. No obvious gross or histopathological changes were observed. The Panel noted the high number of animals that died during the study, therefore the results of the study were of limited value for risk assessment.

Dogs

Groups of three male and three female Beagle dogs were given daily for 90 days gelatine capsules containing 50, 250 and 1,250 mg/kg bw phosphated distarch phosphate (E 1413) (Documentation provided to EFSA n. 22). No effects were observed on body weight, food consumption and behavioural reactions. The results of haematology, clinical blood chemistry, urine analyses and liver function tests showed no differences between the groups. Gross or histopathologic findings were unaffected. Organ weight data and organ-body weight ratios did not reveal any significant differences.

Pigs

Groups of eight Pitman–Moore miniature pigs were weaned at 3 days of age, and fed formula diets containing 5.4% unmodified starch or 5.6% phosphated distarch phosphate (treatment with 4.8% sodium tripolyphosphate and 0.59% sodium trimetaphosphate, both on a dry weight basis; residual phosphorus 0.4%; DS 0.02) for 25 days (Anderson et al., 1973a,b cited in JECFA, 1982e). Growth was normal during the test period. At termination of the study, biochemical analyses of blood (haemoglobin) and serum (cholesterol, triglyceride, calcium, phosphorus, alkaline phosphatase, urea nitrogen, total protein, albumin and globulin) were similar for test and control animals. Relative organ weight as well as carcass composition (water, fat, protein, ash, Ca, PO₄, Na, Mg) and liver composition (water, fat, protein and ash), were similar for test and control animals.

4.2.2.5. Acetylated distarch phosphate (E 1414)

Rats

Groups of 10 male and 10 female rats (CIVO colony, Wistar derived) were given 25% and 50% of acetylated distarch phosphate (cross-linked with 0.02% phosphorus oxychloride and acetylated with 8% acetic anhydride; acetyl content 2.33%) in the diet (equal to 30,000 and 60,000 mg/kg bw per day) for 7 days (Documentation provided to EFSA n. 23). Thereafter, 4% cellulose was added in the diet for a further 3 days. Body weights were slightly reduced in both sexes at the 50% level after 7 days. Faecal dry matter was increased in all test groups. Moderate diarrhoea occurred only at the 50% level in both sexes and was unaffected by the feeding of additional cellulose in the diet. No loss of hair was noted.

Groups of 10 male and 10 female rats (CIVO colony, Wistar derived) were fed 0%, 25% and 50% of acetylated distarch phosphate (cross-linked with 0.02% phosphorus oxychloride and acetylated with 8% acetic anhydride; acetyl content 2.33%) in their diet (equal to 22,500 and 45,000 mg/kg bw per day; applying the default value of 0.09 for the conversion of absolute concentrations in feeding studies with subchronic duration in rat) for 8 weeks (Documentation provided to EFSA n. 23). Differences in body weights were not statistically significant. At the 50% treatment group, body weights of males were slightly lower compared to the control and the 25% groups. The water content of the faeces was higher in males but not in females. Faeces dry matter was increased in both sexes at the higher level tested and slightly so at the 25% dietary level. The incidence of diarrhoea was insignificant. A dose-related increase in caecal weight occurred in both sexes. Histological examination showed no abnormalities compared to the control.

Pigs

Groups of four male and four female pigs were given 0%, 35% or 70% of acetylated distarch phosphate (equal to 8,750 and 17,500 mg/kg bw per day; applying the default value of 0.025 for the conversion of absolute concentrations in feeding studies with pigs, according to WHO, 2009) in their diet over 14.5 weeks (Shillam et al., 1971; cited in JECFA, 1982b). Growth rate and food consumption were satisfactory. Haematology, blood chemistry and urinalysis revealed no treatment-related abnormalities.

Ophthalmoscopy showed no abnormalities associated with the test substance. Organ weight, gross and histopathological examination revealed no abnormalities in the test or control groups. Three pigs in the higher dose test group died suddenly at various intervals during the study without any evidence pointing to the cause of their death. In one of these three pigs, evidence of neurological disorders was observed before death. The neurological disorders were also observed in one of the animals in the 35% group, although in this case the animal recovered. No histopathological evidence of nervous system involvement was noted in these two or in any other animal. The Panel noted that because of neurological disorders which cannot be explained, this study cannot be used for evaluation.

In a further study, groups of eight pigs were fed 0%, 5%, 15% and 25% acetylated distarch phosphate (equal to 1,250, 2,500 and 6,250 mg/kg bw per day; applying the default value of 0.025 for the conversion of absolute concentrations in feeding studies with pigs, according to WHO, 2009) in the diet for 14 weeks (Shillam et al., 1973; cited in JECFA, 1982b). No effect on growth, food consumption, haematology or biochemistry was observed. One pig (treatment group not specified) died of unknown cause. No significant abnormalities were found at post mortem, but histological examination was not performed, except in the animal which died.

Hamsters

Groups of 10 male and 10 female Syrian golden hamsters, weighing 30–40 g, were fed a diet containing either 30% acetylated distarch phosphate or 30% untreated starch for 30 days (Documentation provided to EFSA n. 26). Hamsters fed the test diet showed a slightly lower daily intake (no statistics reported), but the daily body weight gain was comparable or slightly higher than that of the control. No effects were observed in haematology, clinical chemistry or urine analysis data. Histopathological evaluation of liver and kidney showed no treatment-related effects.

4.2.2.6. Acetylated starch (E 1420)

Rats

Groups of 10 male and 10 female rats (CIVO colony, Wistar derived) were given 25% and 50% of starch acetate (prepared with 5% acetic anhydride; acetyl content 1.98%) in a low residue diet (equal to 30,000 and 60,000 mg/kg bw per day) for 7 days (Documentation provided to EFSA n. 23). Thereafter, 4% cellulose was added to the diet for a further 3 days. Body weights were slightly reduced in both sexes at the 50% level after 7 days. Faecal dry matter was increased in all test groups but not in a dose-related manner. Slight diarrhoea occurred only at the 50% level in both sexes and was unaffected by the feeding of additional cellulose in the diet. No loss of hair was noted.

Groups of 10 male rats (Sprague–Dawley) were given diets containing 60% of various starch acetates (using vinyl acetate and acrolein, the degree of acetylation varied from 0%, 1.24%, 2%, 2.56% to 3.25%) for 28 days (equal to 72,000 mg/kg bw per day) (Documentation provided to EFSA n. 16). Weight gain was reduced in groups receiving starch acetates with more than 2% acetylation, but feed efficiency remained unaffected. Diarrhoea occurred at degrees of acetylation of 2% and higher and there was noticeable caecal enlargement at the same levels. No tissue damage or inflammation was noted in association with the diarrhoea.

In a further experiment, groups of 10 male and 10 female rats (CIVO colony, Wistar derived) were given 25% and 50% of starch acetate (prepared with 5% acetic anhydride; acetyl content 1.98%) in the diet (equal to 22,500 and 45,000 mg/kg bw per day) for 8 weeks (Documentation provided to EFSA n. 23). No effects were noted on growth and body weight. Water content of faeces and faecal production, as measured by dry matter content, showed no consistent effects, but there was a tendency towards increased faecal dry matter at the 50% dietary level in both sexes. No diarrhoea was observed in any dietary level. Caecal weight and caecal enlargement occurred in a dose-related manner in all treatment groups. However, histological examination revealed no abnormality of the caeca examined.

In another experiment, potato starch acetate (treated with 5% acetic acid anhydride; acetylated to 1.36%) was fed for 13 weeks to groups of 10 male and 10 female Wistar rats at levels of 5%, 15% and 45% of the diet (equal to 4,500, 13,500 and 40,500 mg/kg bw per day) (Documentation provided to EFSA n. 27). The 5% level was fed for only 4 weeks. No animals died. Growth rates and haematological findings were not significantly affected. The relative organ weights showed relatively small, sometimes statistically significant differences between groups, however these effects showed no dose-response relationship. Caecal weights were slightly higher in treated animals than controls (difference reached statistical significance in males at 45%). No histopathological changes due to starch acetate administration were seen.

4.2.2.7. Acetylated distarch adipate (E 1422)

Rats

In a study focussing on kidney lesions associated with dietary modified starches, rats (six male and female per group, Sprague–Dawley) were given diets containing 30% acetylated distarch adipate (2.14% acetyl) plus 10% unmodified starch or a control diet, consisting of 40% unmodified starch (equivalent to 36,000 mg/kg bw per day), for 30 days (Documentation provided to EFSA n. 28). The mineral mix in the diet was held constant, but Ca, P and Mg varied according to the study design, thus altering the Ca/P content ratios to 1.5/1.36, 1.0/0.96 and 0.5/0.56. The level of magnesium was 0.06% (the required intake). Control groups with the same mineral composition in the diet were used. In addition, at a Ca/P ratio of 0.5/0.56, the magnesium level was lowered to 0.04%. Body weights, clinical and gross observations at necropsy, as well as haematology, were unaffected. At necropsy, organ weights showed no treatment-related effects, except for enlargement of the caecum. Histopathological evaluation of kidney sections from control and test animals revealed a characteristic lesion consisting of mineral deposits in tubules at the corticomedullary junction. This was more common in females than in males. The Panel noted that this lesion is a common finding in older rats, especially in females. There were no other compound-related effects. Histological examination of bone tissue and parathyroid glands showed no effect, even with relatively severely imbalanced Ca/P ratios.

Groups of 15 male and 15 female weanling albino rats, FDRL strain, were fed diets containing either 50% acetylated distarch adipate (equal to 45,000 mg/kg bw per day) or 50% 'thin-boiling' starch as the control diet, for 90 days (Documentation provided to EFSA n. 29). Treated males showed a significantly reduced growth rate (~ 20%). Also food intake and utilisation efficiency was reduced. Relative weights of caeca, empty or full, were higher in both sexes of the test group compared to controls. No treatment-related changes were observed in relative weights of liver and kidneys, or in haematology or blood chemistry analyses. However, female rats on the test diet experienced alkaline urine, compared to the control, at week 6 of the study. No treatment-related adverse effects were observed in the histological sections. Calcification at the corticomedullary junction was observed in six control females and three treated females.

Hamsters

In a special study on kidney lesions associated with dietary modified starch, groups of 10 male and 10 female Syrian golden hamsters, weighing 30–40 g, were fed diets containing either 30% acetylated distarch adipate (E 1422) or 30% untreated starch, for 30 days (Documentation provided to EFSA n. 26). Hamsters fed the test diet showed a slightly lower daily food intake (no statistics reported), but the daily body weight gain was comparable or slightly higher compared to the control. No effects were observed in haematology, clinical chemistry or urinalysis data. Histopathology evaluation of liver and kidney showed no treatment-related effects.

Groups of 8 male and 12 female Syrian golden hamsters (weanlings) were fed diets containing either 30% acetylated distarch adipate at 5 different Mg levels (ranging from deficient to excess) or 30% untreated starch, for periods of either 30 or 60 days (Documentation provided to EFSA n. 30). The diets contained Ca (0.51%), P (0.4%) and Mg (levels of 0.017%, 0.06%, 0.09%, 0.12% or 0.21%; 0.06% is the normal requirement). Half of the animals (4 male/6 female per group) were sacrificed at day 30, and the remaining animals at day 60. At necropsy, animals on test diet showed increased caecal weight. No weight difference was observed for liver and kidney. Haematological studies showed no compound-related effects. Histological sections from kidneys showed mild tubular dilation and small cortical scarring after 30 days in two animals fed 0.017% Mg. After 60 days, in the Mg-deficient diet the effects were more severe, as in nine animals these were judged mild, while in single animals these were judged moderate and severe. No effects were observed in the Mg-excess diet, except effects in one animal in the 0.09% Mg group, judged mild (tubular dilation and small cortical scarring) after 30 days. The dietary magnesium levels or the severity of renal lesions had no impact on kidney magnesium levels, as analysed by neutron activation analysis.

4.2.2.8. Hydroxypropyl starch (E 1440)

Rats

Groups of 10 male and 10 female rats (not further specified) were fed diets containing 0%, 2%, 5%, 10% and 25% (equivalent to 1,800, 4,500, 9,000 and 22,500 mg/kg bw per day) of highly

modified starch (25% propylene oxide) and 25% unmodified starch for 90 days (Kay and Calandra, 1961; cited in JECFA, 1982c). No systemic toxicity was noted. There were no adverse effects regarding mortality, urinalysis or haematology at any treatment level. There was slight reduction in growth rate at the highest dietary level, with lower food utilisation and without an equivalent increase in food consumption. Mild diarrhoea occurred at the 25% dietary level. No adverse effects occurred at any other level. At autopsy, there were no significant differences in the organ weights for liver, kidney, spleen, gonad, heart or brain. Gross and histopathological examination of all major tissues revealed no abnormalities due to the feeding of highly modified starch.

In another experiment, groups of 10 male and 10 female Wistar rats were maintained for 90 days on diets containing 0%, 5%, 15% and 45% (equivalent to 4,500, 13,500 and 40,500 mg/kg bw per day) of low modified starch (5% propylene oxide) (Documentation provided to EFSA n. 31). Body weights did not differ significantly from controls but were consistently lower in male rats only. Food efficiency was similar in all groups. Haematological findings at 12 weeks were comparable for all groups. Caecal enlargement was seen at the 45% level and very slightly at the 15% level. Moderate diarrhoea was observed in both sexes throughout the first 4 weeks at the 45% hydroxypropyl starch level (only slight after 84 days). No histopathological abnormalities related to the test substance were detected in any major organs. The enlarged caeca showed no evidence of inflammation or changes in the muscular tissue.

The effect of hydroxypropyl starches (DS 0.025–0.106) on caecal size and content constituents was studied in a series of experiments over a period of 10 days to 3 months in male Wistar rats (Leegwater et al., 1974). The amount in the diet varied from 10% to 50% (equivalent to 9,000 and 36,000 mg/kg bw per day). The severity of diarrhoea, as well as relative caecal weights, both filled and empty, increased with increasing concentrations of hydroxypropyl starches in the diet, when compared with pregelatinised potato starch controls. Caecal weight also tended to correlate with the DS. Concentrations of sodium, potassium and chloride were decreased in the groups given hydroxypropyl distarch glycerol. The caecum enlargement was reversible after a 4-week recovery period on unmodified, pregelatinised starch.

4.2.2.9. Hydroxypropyl distarch phosphate (E 1442)

Rats

Groups of 10 male rats were fed diets containing 25%, 50%, 75% and 100% hydroxypropyl distarch phosphate (17%, 34%, 51% or 68% as carbon source) for 28 days (equivalent to 30,000, 60,000, 90,000 and 120,000 mg/kg bw per day) (Documentation provided to EFSA n. 32). Sucrose served as a control and was used to complete the diet up to 100%. At the highest levels tested, growth and body weights were reduced compared with controls. For the same levels, the relative liver weights were slightly increased compared with controls fed food grade unmodified starch. The relative organ weights of empty caeca were increased at all levels tested. No histological abnormalities were seen in heart, liver, spleen, kidney and caecum.

Groups of 15 male and 15 female weanling rats (FDRL–Wistar) were fed diets containing 5%, 10% or 25% of starch modified with 10% propylene oxide (equivalent to 4,500, 9,000 and 22,500 mg/kg bw per day), or 25% unmodified starch, for a period of 90 days (FDRL, 1973; cited in JECFA, 1975b). Four rats died during the test period, but deaths were judged by the authors to be no treatment-related. At the highest level of intake of the modified starch, the faeces were soft and bulky during the first 7 weeks of the study, but normal for the rest of the test period. Growth, food intake and food efficiency of all groups was normal, with the exception of a slight decrease in feed efficiency in males in the 25% modified starch group. Haematological, biochemical and urine analysis were within normal limits. At autopsy, absolute and relative organ weights of test and control animals were comparable, with the exception of the caecum. Full caecum weights showed a treatment-related response, however, in the case of empty caeca, significant increase in weight was only observed in males on the 25% diet. Histopathological examination showed that several rats in the test groups had mineralisation of the renal pelvis (5% group: 18/30, 10% group: 20/30, 25% group: 22/30). No other compound-related changes were observed, with the exception of a slight thinning of the caeca, which was not accompanied by histopathological changes.

Groups of 15 male and 15 female rats were fed diets containing 0%, 5%, 10% and 25% of a modified starch prepared by treating cornstarch with 0.1% phosphorus oxychloride and 5% propylene oxide (hydroxypropyl DS 0.07) (equivalent to 4,500, 9,000 and 22,500 mg/kg bw per day) for 90 days (Documentation provided to EFSA n. 33). General condition, growth, food intake and efficiency,

haematology, serum chemistry and urine analyses were not affected at any dietary level. Diarrhoea did not occur, but the water content of the faeces and the amount of faeces dry matter per 100 g food consumed was increased at the 10% and 25% feeding level. The caecal weights, both filled and empty, were distinctly increased only in the 25% diet group in both sexes. Males of this group also showed slightly decreased relative weights of the testes. Macroscopically, no compound-related differences were observed amongst the various groups.

4.2.2.10. Starch sodium octenyl succinate (E 1450)

Rats

In a 8-week study, groups of 12 weanling albino rats (6/sex) were maintained on diets containing 64% carbohydrate ingredients consisting of 29% cellulose, with the remaining 35% consisting of starch sodium octenyl succinate, or cornstarch as a control (Documentation provided to EFSA n. 34). Water was provided *ad libitum*. Body weight and feed consumption were measured weekly: the animals were also observed for behaviour and general physical condition, and complete blood counts and blood sugar and non-protein nitrogen concentrations were measured at the end of the study. Rats fed the substituted starch showed a slightly slower growth rate than control rats fed cornstarch. The decreased growth rate was associated with decreased food consumption. Efficiency of food utilisation was not affected by the test compound.

Rats (Charles River) received a diet containing 6%, 12% or 30% starch sodium octenyl succinate (plus cornstarch at a 30% level of the diet) or 30% cornstarch and were allowed to breed twice (Buttolph and Newberne, 1980). No data and information on reproduction were presented. The animals were allowed to mate twice. The F1b generation was maintained on the same test diet as the parents and used for the study. One hundred weanling rats (equally divided by sex) were used for the 6% and 12% groups, and 120 weanling rats (equally divided by sex) were used for the 30% starch sodium octenyl succinate group and 30% cornstarch group. Twenty animals from the 30% starch sodium octenyl succinate and control groups were killed at 30 days post-weaning, and the remainder of the animals killed 90 days post-weaning. Body weights and food intake were measured during the course of the study. Clinical chemistry (sodium, potassium, chloride, glucose, BUN, magnesium, alkaline phosphatase, serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), calcium, phosphates, total protein and albumin), haematology (RBC, WBC, haematocrit, haemoglobin, total protein and differential blood count) and urinalysis (pH, total protein, glucose, ketone, occult blood, sodium, potassium, creatinine, calcium and magnesium) was carried out in selected animals at the termination of the study. All animals killed at intervals or at the termination of the study were subjected to complete necropsy. Relative and absolute weights of organs (kidneys, liver, spleen, brain, thymus, testes or uterus) were determined, and a complete histopathological evaluation of the principal organs and tissues was made. There was no significant effect on growth rate. Serum chemistry and haematology were within normal levels and showed no compound-related effects. Urine chemistry showed higher concentrations of urinary calcium and magnesium in females but not in males. Relative organ weight data showed a trend for increased liver and kidney weight with increased concentration of the substituted starch in the diet. There was an increased caecal weight in the animals fed 30% starch sodium octenyl succinate in both sexes after 30 days, but this was only observed in females after 90 days on the test diet. The only significant histological finding was an incidence of corticomedullary mineralisation in the kidneys. The effect was more severe in females than in males, and occurred in animals fed either the modified or unmodified starch.

In a 90-day study (Unilever, 1984; cited in JECFA, 2015), groups of 10 male and 10 female Colworth–Wistar rats were fed one of the following diets: an 'in-house' developed purified diet containing 10% fat, 25% protein, 0.05% magnesium and 30% unmodified starch (control diet 1); an Environmental Safety Laboratory (ESL)-modified American Institute of Nutrition (AIN-76) diet containing 5% fat, 20% protein, starch in replacement of sucrose, 0.2% magnesium and 30% unmodified starch (control diet 2); the ESL-modified AIN-76 diet supplemented with a trace element mixture and 30% unmodified starch (control diet 3); or the same diets but replacing the 30% unmodified starch with OSA-modified starch (test diets 1, 2 and 3). Therefore, the control diets for each of the OSA-modified test groups contained unmodified starch. The modified starch diets provided approximately 37,000 mg/kg bw per day of OSA-modified starch. Animals were routinely monitored for clinical signs, body weights and feed intake. The parameters evaluated included serum biochemistry, urine analysis, organ weights, liver composition and histopathology.

There were no differences in body weight gain, feed consumption, plasma chemistry measurements or urine analysis parameters when comparing animals on the test diets (OSA-modified starch) with those on the corresponding basal control diets. The fact that the feed intake was not reduced in this study supports the view that the reduction in feed intake noted in the first study (Documentation provided to EFSA n. 34) was due to the poor palatability of the diet. Similarly, no significant test article-related changes in liver, kidney or caecum weights were observed when comparing the animals on the test diets with those on the corresponding basal control diets. Although the liver weights in male rats fed test diet 3 (the modified AIN-76 diet supplemented with a trace element mixture and OSA-modified starch) were lower than those of the corresponding control group, the authors noted that the liver weights in this control diet 3 group were significantly higher than in the other control groups. Increased liver weights were seen in animals fed the AIN-76 diets and increased kidney weights were observed in those fed the 'in-house' developed purified diets. However, the higher kidney weights observed in rats fed the 'in-house' purified diets compared with the AIN-76 diets were considered to be related to corticomedullary nephrocalcinosis. All female animals fed the 'in-house' purified diets exhibited corticomedullary nephrosclerosis, and it was noted that the inclusion of OSA-modified starch did not influence its severity. Similar effects were not apparent in male rats.

The authors concluded that the inclusion of OSA-modified starch in the diet of rats for 90 days at a concentration of 30% (approximately 37,000 mg/kg bw per day) did not adversely affect any parameter examined, when compared with the control unmodified starch (Unilever, 1984; cited in JECFA, 2015). The Panel agreed with this conclusion.

4.2.2.11. Acetylated oxidised starch (E 1451)

In a 14-day range-finding study, groups of five male Wistar rats received a diet containing 0%, 10%, 30% and 50% acetylated oxidised starch (equivalent to 0, 5,000, 15,000 or 25,000 mg/kg bw per day) (Documentation provided to EFSA n. 35). The test was performed according to good laboratory practice (GLP). One group received basal diet and another received 30% unextruded acetylated oxidised starch (equivalent to 25,000 mg/kg bw per day). No deaths or differences in appearance or behaviour were observed. The difference in mean body weight was not statistically significant between the groups. Also, food intake and food efficiency were unaffected. Rats given 50% acetylated oxidised starch had soft faeces from day 2 onwards. The absolute and relative caecal weights (filled and empty) were increased in animals given 30% and 50% acetylated oxidised starch, as well as 30% unextruded acetylated oxidised starch. At the end of the study, macroscopic examination showed dilated caeca in animals fed 30% and 50% acetylated oxidised starch. The caeca of animals given 50% acetylated oxidised starch were flabby. The no-observed-effect level (NOEL) was 10% acetylated oxidised starch in the diet, equivalent to 5,000 mg/kg bw per day, on the basis of increased caecal weights and dilatation of the caeca.

In the following 90-day study, groups of 10 male and 10 female Wistar rats received diets containing 0%, 5%, 10% or 30% acetylated oxidised starch (equal to 0, 3,000, 5,900 and 18,000 mg/kg bw per day for males and 0, 3,400, 6,600 and 20,000 mg/kg bw per day for females). The test was performed according to GLP and OECD Test Guideline (TG) 408 (OECD, 1981). Condition and behaviour were monitored twice daily on working days and once daily at weekends and holidays, and all clinical signs were noted. Ophthalmoscopy was carried out on all rats before the study and on all rats in the 0% or 30% groups at the end of the study. Body weight, food intake and food efficiency were recorded weekly. Haematological examinations and clinical chemistry were performed on all rats at termination. All rats were examined macroscopically and organs from all rats at 0% or 30% were examined microscopically. In addition, the kidneys, liver, lungs and gross lesions from all animals at the 5% and 10% level, and the urinary bladders of all male animals at the 5% and 10% level, were examined microscopically.

No deaths were seen. No clinical signs of toxicity or behavioural abnormalities were observed. The ophthalmoscopic parameters were unaffected. Body weight, food intake and food efficiency did not show treatment-related differences among the groups. Haematology did not reveal dose-related changes in red or white blood cell variables or in clotting potential. Blood biochemical characteristics were comparable in all groups. Clinical chemistry and urinary analysis revealed no significant changes. The absolute and relative weights of the filled and empty caecum were increased in rats in the 30% group. The mean relative weights of the adrenals and kidneys of males at the lowest concentration group were significantly increased, but no change was seen at the two higher concentrations or in organ weights in females. Therefore, the changes in the weights of the adrenals and kidneys in males

at 5% were considered to be of no biological significance. Dilated caecum was observed in one male rat at 30%.

At microscopic examination, focal hyperplasia of the urinary bladder epithelium was observed in four male rats in the 30% group. This effect was not seen at lower concentrations, in controls, or in females fed at the 30% level. In females, at the 30% level, the incidence of hyperplasia of transitional epithelium in the kidney was higher compared to the control. However, the effect was not statistically significant. In addition, the incidence of mineralisation was slightly higher in animals fed the 30% acetylated oxidised starch diet. Mineralisation occurred not only in the pelvic area but also in the cortex and the medullary zone. The frequency of small aggregates of reticuloendothelial cells in the liver showed no dose–response relationship, and the increase reached statistical significance in males in the 5% group. This effect is a common finding in rats of the strain and age used and was considered by the authors to be an incidental finding. The NOAEL in this study was considered to be 10% in the diet, equal to 5,900 mg/kg per day, on the basis of microscopic changes in the kidney and urinary bladder epithelium (Documentation provided to EFSA n. 35).

4.2.2.12. Starch aluminium octenyl succinate (E 1452)

Groups of 10 male albino rats were fed 1.5 or 3.0 g of an aluminium octenyl succinate derivative of a waxy 'thin-boiling' starch every day for 4 weeks (FDRL, 1961; cited in Nair and Yamarik, 2002). A control group was fed the non-modified starch. Weight gain, behaviour and growth were comparable among test and control rats.

Groups of six male and six female weanling albino rats (strain not specified) were given diets containing 1% or 10% starch aluminium octenyl succinate for 8 weeks. Because no toxic signs were observed in the first 4 weeks of the study, the 1% dose was increased to 25% starch aluminium octenyl succinate for the remaining 4 weeks. Control rats were fed cornstarch at 35%. Body weight and feed consumption, as well as behaviour and conditions, were not affected by the treatment. Haematology (complete blood counts and blood sugar and nonprotein nitrogen) was similar between the treatment and the control group (Documentation provided to EFSA n. 34).

4.2.2.13. Summary

Short-term and/or subchronic (90-day) studies in rats were available for all modified starches, except monostarch phosphate (E 1410), but occasionally also studies in dogs, pigs or hamsters were available. The modified starches were given at dietary levels up to 70%. The test duration was up to 90 days. Effects on body weight and feed consumption were not observed up to dietary levels of 25%. Caeca weights of treated animals were not different from those of controls.

Caeca weights were increased at exposure levels of 30% and higher, but without histopathological changes. The only significant histopathological change was the presence of pelvic and/or corticomedullary mineralisation in the kidneys, which was observed with modified as well as unmodified starches, and occurred more pronounced in females than in males.

In a 90-day study with acetylated oxidised starch (E 1451) in rats, a NOAEL of 10% in the diet, equal to 5,900 mg/kg bw per day, was identified based on microscopic changes in the kidneys and urinary bladder epithelium, which were observed at 18,000 mg/kg bw per day, the following dose in this study.

4.2.3. Genotoxicity

Evaluation of genotoxicity of modified starches was performed *in silico*, since no genotoxicity studies were available. On this basis, identification of structural alerts for genotoxicity for distarch phosphate, phosphated distarch phosphate, distarch adipate, acetylated distarch phosphate, acetylated starch, acetylated distarch adipate, hydroxypropyl starch, hydroxypropyl distarch phosphate and starch sodium octenyl succinate was performed using the OECD QSAR Toolbox (version 3.3.5.17).

No relevant structural alerts for genotoxicity (profilers 'Alerts for Ames, chromosomal aberrations and micronuclei by Oasis 1.2' and 'in vitro (Ames test) and in vivo mutagenicity (micronucleus) by ISS') were highlighted for distarch phosphate, phosphated distarch phosphate, hydroxypropyl starch, hydroxypropyl distarch phosphate and starch sodium octenyl succinate.

The alkyl hydroperoxide structural alert was triggered by distarch adipate. This structure (highlighted in Figure 6) is known to generate, through enzymatic and non-enzymatic cleavage, alkoxyl, peroxy and hydroxyl radicals (RO $\cdot$, ROO $\cdot$, HO $\cdot$, respectively), which may elicit DNA damage (Kovacic and Jacintho, 2001).

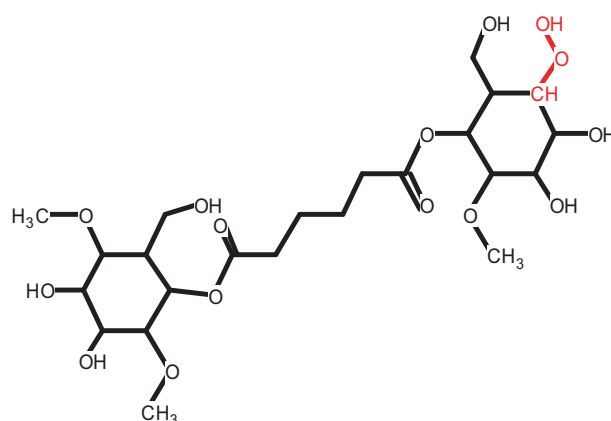


Figure 6: Alerting alkyl hydroperoxide group present in distarch adipate

However, the Panel noted that in general, the genotoxic effects induced by this structural alert are more relevant *in vitro* than *in vivo*, since cell antioxidant defences and the ability of the cell to cope with the formation of reactive oxygen species (ROS) are more efficient in the whole organism.

An alert for acylation (by S_N2 mechanism) was highlighted by one profiler (Oasis v.1.3) for acetylated distarch phosphate, acetylated starch and acetylated distarch adipate. The structural alert detected, *specific acetate esters*, refers to the presence of an acetate ester with possible enhanced reactivity due to the presence of an electron-withdrawing group attached to the carbon atom at β -position towards the ester oxygen (represented by the oxygen pointed by the arrow in Figure 7).

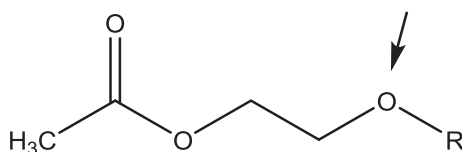


Figure 7: Alerting acetate ester group present in the three acetylated starches

The Panel noted that the same alert is not detected by the other endpoint specific profiler (*in vitro* mutagenicity (Ames test) alerts by ISS), and that this different outcome points out a not univocal interpretation of the relevance for genotoxicity of this specific structural feature, as also reported in the alert accompanying explanations: 'Such esters belong to a very limited and specific scope of chemicals of different structures, since acetate esters are, in most cases, non-mutagenic. Hence, no generalised chemical mechanistic schemes, associated with the positive bacterial mutagenicity (*in vitro* genotoxicity) of such acetate esters can be inferred, and different mechanisms may operate'.

Indeed, a search in an external database (ISSSTY) did not highlight a correlation between the alert *specific acetate ester* and genotoxicity (Appendix F).

One additional structural alert 'Hacceptor-path3-Hacceptor' was detected by the profiler '*in vivo* mutagenicity (micronucleus) by ISS', for all listed compounds. However, this alert, which is present in the glucose molecule, is considered not relevant, as it refers to non-covalent binding to DNA or proteins as a result of the presence of two bonded atoms connecting two hydrogen bond acceptors and as its positive predictivity is quite low, ranging from 'none' (34%) to just 63% depending on the database, with a high incidence of false positives (Benigni et al., 2010, 2012).

Overall, the Panel concluded that the *in silico* analysis of the substructures of modified starch moieties did not identify any relevant alert for genotoxicity, and concluded that modified starches do not raise concern for genotoxicity.

4.2.4. Chronic toxicity and carcinogenicity

No data were available for oxidised starch (E 1404), monostarch phosphate (E 1410), distarch phosphate (E 1412), hydroxypropyl starch (E 1440), acetylated oxidised starch (E 1451) and starch aluminium octenyl succinate (E 1452).

4.2.4.1. Phosphated distarch phosphate (E 1413)

Rats

Groups of 30 male and 30 female rats (Wistar-derived) were fed phosphated distarch phosphate (maize starch 'white milo', cross-linked with sodium trimetaphosphate up to 0.04% introduced phosphorus and esterified with sodium tripolyphosphate up to a total content of 0.35% bound phosphorus, commercial name Snow Flake 4832) at dietary levels of 0%, 5%, 10% and 30% (equivalent to 0, 2,500, 5,000 and 15,000 mg/kg bw per day) for 104 weeks (documentation provided to EFSA n. 36; de Groot et al., 1974). No treatment-related effects were noted on general appearance, behaviour or mortality. Food intake, growth rate and food efficiency in treated animals were comparable to controls. Haematology, clinical chemistry and urine analysis revealed no consistent or dose-related differences between the test groups and the controls. Relative organ weights were comparable to those of the controls, except for significantly decreased spleen weight in males and significantly increased spleen and kidney weights in females fed at 30%. These changes were not associated with any gross pathological findings. Caecal weights were not increased. Histological examination did not reveal any distinct compound-related changes. The study did not reveal any indication of carcinogenicity. The authors stated that 'in comparison with the controls, the males fed the 30% level of the modified starch showed a slightly increased degree and incidence of focal hyperplasia of the renal papillary and pelvic epithelium, accompanied by calcified patches in the underlying tissue. The hyperplastic and calcified tissues often protruded into the renal pelvis and were localised most often in the papilla near the junction of the papillary and pelvic epithelium. This lesion was seen to a slight or moderate degree in both sexes at most levels including the controls but was more pronounced and of higher occurrence in males at the highest dose level.'

4.2.4.2. Acetylated distarch phosphate (E 1414)

Rats

Groups of 30 male and 30 female rats (Wistar-derived) were fed acetylated distarch phosphate (potato starch cross-linked with 0.02% phosphorus oxychloride and acetylated with 8% acetic anhydride; acetyl content 2.33%) at dietary levels of 0%, 5%, 10% and 30% (equal to 0, 2,500, 5,000 and 15,000 mg/kg bw per day) for 104 weeks (Documentation provided to EFSA n. 37; de Groot et al., 1974). No treatment-related effects were noted on general appearance, behaviour or mortality. Food intake, growth rate and food efficiency in treated animals were comparable to controls. The final body weight was slightly reduced and was approx. 10% lower, significant at least in males at 30%. Haematology, clinical chemistry and urine analysis revealed no consistent or dose-related differences between the test groups and the controls. Females exhibited a dose-related increase in relative adrenal weight (significant at 30%). There was a dose-related increase in the caecal weight in both sexes at the 30% level, but only in males at the 10% level. The caecal enlargement was attributed to an adaptive response (fermentation) to the presence of indigestible material, rather than to a pathological response. All other organ weights showed no treatment-related changes. The only treatment-related effect that was observed histologically was a kidney lesion which occurred at a higher incidence in the high-dose males. The lesion consisted of suburothelial deposits of calcium accompanied by focal hyperplasia of the epithelium of the renal pelvis. No treatment-related effect was observed on the pattern of neoplasm development.

In a study focussing on kidney lesions associated with dietary modified starches, groups of 25 female Sprague–Dawley rats were fed diets containing either 30% acetylated distarch phosphate (equivalent to 15,000 mg/kg bw per day) or 30% unmodified starch, used as a control; this comprised a 1-year study in weanling rats (Experiment I) and a separate 9-month study utilising 9-month-old rats (Experiment II) (Hodgkinson et al., 1982). The concentration of Ca, P and Mg in the diet was 1%, 0.8% and 0.15%, respectively. Body weight, food consumption, urine volume, urine pH and crystal content or faecal mineral content showed no differences between treated and control animals in both experiments. At necropsy, caecal weight was significantly increased, but no other treatment-related effects on relative organ weights were observed. No treatment-related histopathological effects were observed in the uterus or lower urinary tract, liver, parathyroid, caecum or ovaries in either experiment. Histopathological examination of kidney sections demonstrated the presence of treatment-related pelvic nephrocalcinosis. An apparent correlation was observed between the increased incidence of pelvic nephrocalcinosis, increased accumulation of calcium in the kidney and increased urinary

excretion of calcium. Residues of calcium in kidney tissue were significantly higher in the test groups than in the control.

The effects of mineral deposition in the renal pelvis of rats were reviewed in 1977 by an expert (Documentation provided to EFSA n. 38). After an extensive evaluation, it was concluded that pelvic nephrocalcinosis, corticomedullary nephrocalcinosis, acute tubular nephropathy and calculus formation are manifestations of mineral imbalance and are of relatively common occurrence in untreated laboratory rats (particularly in older animals) (FASEB, 1979). The Panel agreed with this conclusion.

4.2.4.3. Acetylated starch (E 1420)

Rats

Groups of 30 male and 30 female rats (Wistar-derived) were fed starch acetate (potato starch treated with 5% acetic anhydride; acetyl content 1.98%) at dietary levels of 0%, 5%, 10% and 30% (equivalent to 0, 2,500, 5,000 and 15,000 mg/kg bw per day) for 104 weeks (Documentation provided to EFSA n. 39; de Groot et al., 1974). No treatment-related effects were noted on general appearance, behaviour or mortality. Food intake, growth rate and food efficiency, as well as body weight of treated animals were comparable to controls. Haematology, clinical chemistry and urine analysis revealed no consistent or dose-related differences between the test groups and the controls. There was a dose-related increase in the caecal weight in both sexes at the 30% level, but in males only, at the 10% level. The caecal enlargement was attributed to an adaptive response to the presence of indigestible material, rather than to a pathological response. All other organ weights showed no treatment-related changes. The only treatment-related effect that was observed histopathologically was a kidney lesion which occurred at a higher incidence in the high-dose males. The lesion consisted of suburothelial deposits of calcium accompanied by focal hyperplasia of the local epithelium of the renal pelvis. Based on the renal lesions, the Panel identified a NOAEL of 5,000 mg/kg bw per day. No treatment-related effect was observed on the pattern of neoplasm development.

4.2.4.4. Acetylated distarch adipate (E 1422)

Rats

Groups of 30 male and 30 female rats (Sprague–Dawley derived) were fed acetylated distarch adipate (modification of maize starch with acetic anhydride as stabiliser and adipic acid as cross-linking agent; acetyl content 2.5%) at dietary levels of 62% (equal to 31,000 mg/kg bw per day) for 104 weeks (Truhaut et al., 1979). Unmodified starch at 62% served as control. Body weight was significantly lower in treated males and females compared to control. However, femur measurements indicated no accompanying differences in skeletal growth and, at autopsy, control rats contained markedly greater adipose deposits than those found in the treated rats of either group. Haematology, serum biochemical analyses, bacteriological examinations and organ-weight determinations showed no significant differences of pathological interest (e.g. elevated SGOT) between control and treated animals. Histological examination of the main organs did not reveal any significant differences between control and treated groups for either non-tumorous lesions or tumours. In the case of the kidney, hyperplasia of the kidney urothelium, sometimes accompanied by calcification, was observed in both control and test groups, but neither the incidence nor the severity of these effects were considered by the authors to be treatment-related. However, independent reviewers of the data concluded that in female rats, the incidence of epithelial hyperplasia was greater in the rats fed modified starch. The Panel agreed with the conclusions of the independent reviewers.

Groups of 25 female Sprague–Dawley rats were fed diets containing either 30% acetylated distarch adipate (equivalent to 15,000 mg/kg bw per day) or 30% unmodified starch (control) in a 1-year study with weanling rats (Experiment I) and a separate 9-month study utilising 9-month-old rats (Experiment II) (Hodgkinson et al., 1982). The calcium concentration in the diet was ~ 1%, phosphorus ~ 0.8% and magnesium ~ 0.15%. Urinary calcium concentration and total daily output were significantly increased in animals on the test diet (Experiments I and II), but only minor differences were seen in phosphorus, oxalate, magnesium and creatinine excretion. No significant effects were observed on body weight, food consumption, urine volume, urine pH and crystal content or faecal mineral content in animals on the test diet. At necropsy, relative organ weights showed no differences between the groups, except for caecal enlargement. No treatment-related histopathological effects were observed in the uterus or lower urinary tract, liver, parathyroid, caecum or ovaries in either experiment. Histopathological examination of kidney sections demonstrated the presence of treatment-related pelvic nephrocalcinosis. An apparent correlation was observed between the increased incidence of

pelvic nephrocalcinosis, increased accumulation of calcium in the kidney, and increased urinary excretion of calcium. Residues of calcium in kidney tissue were significantly higher in the test groups than in the controls.

4.2.4.5. Hydroxypropyl distarch phosphate (E 1442)

Mice

Groups of 75 male and 75 female Swiss albino SPF mice were fed a diet containing 55% hydroxypropyl distarch phosphate (equivalent to 27,500 mg/kg bw per day) or a control diet containing 55% pregelatinised potato starch for 89 weeks (Documentation provided to EFSA n. 40; Til et al., 1986). Observations were made on growth and appearance, haematology, blood biochemistry, urine composition, organ weights, mortality and gross and microscopic pathology, with special attention being given to the kidney and bladder. In week 80, 10 mice/sex per group were killed and necropsied. A thorough necropsy was also performed on those animals found dead or moribund. After 89 weeks, all survivors were killed and subjected to necropsy.

Loose stools and slight diarrhoea was observed in 12% of the males and 5% of the females. In the control group, it was slightly lower (males: 4%, females: 3%). Loss of body weight prior to death was observed in about 25% of male control animals, whereas in the other groups, at most 10% of the males was found to lose weight. Such differences between groups were not noticed in females. The death rate in the other groups was quite normal for the strain of mice used, except for a fairly high mortality in males of the control group between week 39 and week 65. The body weights of the group fed hydroxypropyl distarch phosphate were significantly decreased in males from week 16 to 48 and in females from week 40 onwards, compared to the control. Water intake was increased in both males and females of the treated group to ~ 100% in week 86. Haematocrit was reduced in both sexes at week 40 but not at week 78. Clinical chemistry was unaffected. In male mice, a higher incidence of amorph material in the urine was observed, and the rate of turbid urine was higher. Examination of the urine sediment by IR spectroscopy revealed that the sediment consisted of nearly 100% protein. The caecum weight of treated animals, with or without contents, was statistically higher compared with the control group. Similar differences were found for the colon. Histopathological evaluation revealed an increase in the incidence of intratubular mineralisation in the kidneys of treated male and female animals. There was no evidence of carcinogenicity (Documentation provided to EFSA n. 40; Til et al., 1986). The Panel agreed with this conclusion.

4.2.4.6. Starch sodium octenyl succinate (E 1450)

Rats

Male and female Colworth–Wistar rats (52 of each sex/group) were fed OSA-modified starch in the diet at concentrations of 0%, 5%, 12.5% or 30% (equivalent to 0, 2,800, 7,100 and 17,000 mg/kg bw per day for males and 0, 3,500, 8,800 and 21,000 mg/kg bw per day for females) for 120 and 116 weeks, respectively (Parish, 1987; cited in JECFA, 2015). The rats were 4 weeks old at the beginning of dosing. Maize starch was added to compensate for the different levels of OSA-modified starch added to the diet. Autopsy was performed when survival was below 25%, which occurred at 116 weeks for females and 120 weeks for males. The addition of OSA-modified starch did not affect mortality. No statistically significant difference in the overall body weight gain was noted in male rats. However, an increase in body weight gain in female rats occurred in the 5% and 12.5% OSA-modified starch group between 0 and 114 weeks. There was no treatment-related change in feed intake, feed efficiency or ophthalmoscopy. The absolute and relative pituitary weights were found to be significantly increased in male rats in the 30% OSA-modified starch group, which was related to pituitary adenoma in those surviving rats. This finding is common in rats of this age and strain.

The author concluded that there was no evidence for carcinogenicity or chronic toxicity of OSA-modified starch when fed to rats at concentrations of up to 30% in the diet, equivalent to 17,000 mg/kg bw per day (Parish, 1987; cited in JECFA, 2015). The Panel agreed with this conclusion.

4.2.4.7. Summary

Two chronic studies (52-week) were available, one with acetylated distarch phosphate (E 1414) and one with acetylated distarch adipate (E 1422) (Hodgkinson et al., 1982). At necropsy, relative organ weights showed no differences between the groups, except for caecal enlargement. Histopathological examination of kidney sections demonstrated the presence of treatment-related pelvic nephrocalcinosis. An apparent correlation was observed between the increased incidence of pelvic

nephrocalcinosis, increased accumulation of calcium in the kidney and increased urinary excretion of calcium.

A carcinogenicity study (89-week) in mice was available for hydroxypropyl distarch phosphate (E 1442) (Documentation provided to EFSA n. 40). There was no evidence of carcinogenicity. This chronic study in mice demonstrated some histopathological changes in the kidneys characterised by intratubular mineralisation, which according to the authors, was of no toxicological significance for the human health.

Carcinogenicity studies in rats were available for phosphated distarch phosphate (E 1413), acetylated distarch phosphate (E 1414), acetylated starch (E 1420), acetylated distarch adipate (E 1422), hydroxypropyl distarch phosphate (E 1442) and starch sodium octenyl succinate (E 1450). There was no evidence of carcinogenicity. The long-term studies in rats did not reveal any significant effects, except for caecal enlargement. As this effect was observed without associated histopathological changes, it was considered to be of no toxicological significance for humans.

Kidney lesions (pelvic and corticomedullary mineralisation) in rats fed high levels (up to 62%; 31,000 mg/kg bw per day) of phosphated distarch phosphate (E 1413), acetylated distarch phosphate (E 1414), acetylated starch (E 1420), acetylated distarch adipate (E 1422) and hydroxypropyl distarch phosphate (E 1442) were observed. The lesions were considered to be associated with an imbalance of Ca/P and Mg in the diet (Newberne and Buttolph, 1979; Documentation provided to EFSA n. 28; Documentation provided to EFSA n. 38; Hodgkinson et al., 1982). As the rat is a particularly sensitive species for PN (Ritskes-Hoitinga et al., 1989, 1991, 1992), while the effect was not observed in the hamster and the pig, the effect was considered to be of no relevance for risk assessment in humans. These renal changes were not observed in rats fed starch sodium octenyl succinate (E 1450) at 30% in the diet for up to 120 weeks (Parish, 1987; cited in JECFA, 2015).

4.2.5. Reproductive and developmental toxicity

No data were available for oxidised starch (E 1404), monostarch phosphate (E 1410), distarch phosphate (E 1412), hydroxypropyl starch (E 1440), hydroxypropyl distarch phosphate (E 1442), acetylated oxidised starch (E 1451) and starch aluminium octenyl succinate (E 1452).

4.2.5.1. Phosphated distarch phosphate (E 1413)

Reproductive toxicity studies

A three-generation study was performed using groups of 10 male and 20 female rats (Wistar-derived) of the P, F1 and F2 generations to produce two successive litters in each generation by mating at weeks 12 and 20 after weaning (Documentation provided to EFSA n. 41; de Groot et al., 1974). A total of 10 males and 10 females of the F1b generation were kept for 3 weeks after weaning and then sacrificed for histopathological studies. The P, F1b and F2b parents were used for determination of implantation sites. The F3b generation was kept for 3 weeks after weaning and then sacrificed for histopathological evaluation. The phosphated distarch phosphate (maize starch 'white milo', cross-linked with sodium trimetaphosphate up to 0.04% introduced phosphorus and esterified with sodium tripolyphosphate up to a total content of 0.35% bound phosphorus, commercial name Snow Flake 4832) was fed at 10% in the diet (equal to 5,000 mg/kg bw per day). The control group was fed unmodified potato starch. No adverse effects were noted regarding appearance, behaviour, body weight, fertility, litter size, resorption quotient, weights of pups and mortality. Caecal weights were not increased, except for the filled caecum weight of F1 parent males. The spleen weight of F3b females was increased significantly ($p < 0.01$). Gross and macroscopical examination did not reveal histopathological changes attributable to the ingestion of this starch.

4.2.5.2. Acetylated distarch phosphate (E 1414)

Reproductive toxicity studies

A three-generation study was performed using groups of 10 male and 20 female rats (Wistar-derived) of the P, F1 and F2 generations to produce two successive litters in each generation by mating at weeks 12 and 20 after weaning (Documentation provided to EFSA n. 41; de Groot et al., 1974). A total of 10 males and 10 females of the F1b generation were kept for 3 weeks after weaning and then sacrificed for histopathological studies. The P, F1b and F2b parents were used for determination of implantation sites. The F3b generation (10 rats/sex) was kept for 3 weeks after weaning and then sacrificed for histopathological examination. The acetylated distarch phosphate

(potato starch cross-linked with 0.02% phosphorus oxychloride and acetylated with 8% acetic anhydride; acetyl content 2.33%) was fed at 10% of the diet (equivalent to 5,000 mg/kg bw per day). No adverse effects were noted with respect to health, behaviour, mortality, growth, fertility, litter size, resorption quotient, weaning weight or mortality of the young. Caecal weight of parent rats fed the modified starch was not increased. Macroscopical examination did not reveal treatment-related effects in F3b rats. Relative thyroid weight in males was decreased ($p < 0.05$) and furthermore, a slightly increased caecum weight in females ($p < 0.05$) was observed. Histopathology did not reveal any treatment-related changes.

Developmental toxicity

No studies available.

4.2.5.3. Acetylated starch (E 1420)

Reproductive toxicity studies

A three-generation study was performed using groups of 10 male and 20 female rats (Wistar-derived) of the P, F1 and F2 generations to produce two successive litters in each generation by mating at weeks 12 and 20 after weaning (Documentation provided to EFSA n. 41; de Groot et al., 1974). A total of 10 males and 10 females of the F1b generation were kept for 3 weeks after weaning and then sacrificed for histopathological studies. The P, F1b and F2b parents were used for determination of implantation sites. The F3b generation was kept for 3 weeks after weaning and then sacrificed for histopathological studies. The starch acetate (potato starch treated with 5% acetic anhydride; acetyl content 1.98%) was fed at 10% in the diet (equivalent to 5,000 mg/kg bw per day). No adverse effects were noted with respect to health, behaviour, mortality, growth, fertility, litter size, resorption quotient, weaning weight or mortality of young animals. Relative caecum weight was significantly increased in P females (empty, $p < 0.05$) and F2 females (filled and empty, $p < 0.05$). Gross pathology of F3a rats revealed a slightly increased kidney weight ($p < 0.05$) and a slightly increased caecum weight ($p < 0.01$) in males. Histopathological examination did not reveal any treatment-related changes.

Developmental toxicity

No studies available.

4.2.5.4. Acetylated distarch adipate (E 1422)

Reproductive toxicity studies

Groups of 10 male and 10 female rats (Sprague-Dawley derived) were selected at random from a concurrent 2-year chronic toxicity study (see Section 4.2.4.4) and, 6 weeks after weaning, were mated to produce F1a and F1b litters (Truhaut et al., 1979). They were fed acetylated distarch adipate (maize starch modified with acetic anhydride as a stabiliser and adipic acid as a cross-linking agent; acetyl content 2.5%) at dietary levels of 62% (equivalent to 31,000 mg/kg bw per day). Unmodified starch at 62% served as a control. After breeding was complete, parents were returned to the chronic study, while 10 male and 10 female rats from the F1b litter were bred to produce F2a and F2b litters. F3a and F3b litters were obtained in a similar way. In each generation, litters from the first mating were sacrificed at weaning, and from the second mating, 6 weeks after weaning, except for the 10 males and 10 females selected for breeding. Prewaning deaths were significantly elevated in offspring from F2b litters for both control and test animals compared to the previous generation, but were within normal limits for the strain. The remaining test parameters (litter size, incidence of stillbirths and sex ratio at weaning) were similar in treated and control animals. Growth was comparable in all groups. Terminal studies of the F3b generation (including histology of the principal organs) did not reveal evidence of anomalies. No detailed information from the histopathological examinations was provided.

Developmental toxicity

No studies available.

4.2.5.5. Starch sodium octenyl succinate (E 1450)

There were no data available from specific studies on reproductive or developmental toxicity for starch sodium octenyl succinate (E 1450). However, there was an investigation of the short-term toxicity in F1 offspring of Fischer 344 rats that had been fed OSA-modified starch from weaning and

throughout mating, gestation and lactation. The F1 offspring were fed the same diets as their dams from weaning and for 30 or 90 days post-weaning (see Section 4.2.2.10). No comment was made regarding any effect on the offspring (Buttolph and Newberne, 1980).

4.2.5.6. Summary

Dietary reproductive toxicity studies in rats were available for phosphate distarch phosphate (E 1413), acetylated distarch phosphate (E 1414), acetylated starch (E 1420) and acetylated distarch adipate (E 1422). No effects on reproductive performance or maternal and developmental toxicity were observed in the three-generation reproductive toxicity studies at dietary levels of up to 62% (equivalent to 31,000 mg/kg bw per day).

No prenatal developmental toxicity studies were available.

4.2.6. Other studies

4.2.6.1. Phosphated distarch phosphate (E 1413)

Human studies

Twelve volunteers consumed on each of 4 successive days, 60 g phosphated distarch phosphate (maize starch 'white milo', cross-linked with sodium trimetaphosphate up to 0.04% introduced phosphorus and esterified with sodium tripolyphosphate up to a total content of 0.35% bound phosphorus, commercial name Snow Flake 4832). No abnormalities were observed (Documentation provided to EFSA n. 42).

In an opinion of the EFSA NDA Panel, the results of one unpublished study with phosphated distarch phosphate were reported (EFSA NDA Panel, 2010). Eleven healthy non-diabetic adults were fed biscuits containing the novel ingredient at various levels (6.8%, 13.6%, 20.4% or 27.1%) vs control biscuits. Over a period of 2 h, the novel ingredient had no effect on glycaemic response at any of the concentrations tested.

4.2.6.2. Acetylated distarch phosphate (E 1414)

Human studies

Twelve volunteers consumed on each of 4 successive days, 60 g acetylated distarch phosphate (potato starch cross-linked with 0.02% phosphorus oxychloride and acetylated with 8% acetic anhydride; acetyl content 2.33%). No abnormalities were observed as regards frequency and amount of faeces, as well as faecal water and lactic acid content. No other adverse effects were noted (Documentation provided to EFSA n. 42).

4.2.6.3. Acetylated starch (E 1420)

Human studies

Twelve volunteers consumed on each of 4 successive days, 60 g starch acetate (potato starch treated with 5% acetic anhydride; acetyl content 1.98%). No abnormalities were observed as regards frequency and amount of faeces, as well as faecal water and lactic acid content. No other adverse effects were noted (Documentation provided to EFSA n. 42).

4.2.6.4. Hydroxypropyl starch (E 1440)

Rats

The effects of hydroxypropyl starches (HPS) of three different degrees of substitution (DS = 0.046, 0.093 and 0.232) on concentration of plasma cholesterol, apparent digestibility of protein, faecal excretion of bile acids, faecal output and caecal pool of organic acids such as acetic, propionic, butyric, lactic and succinic acid were studied in rats (Ebihara et al., 1998). Male rats (n = 6, Wistar strain) were fed a fibre-free, purified diet containing either HPS or gelatinised unmodified potato starch (PS; 100 g/kg) as a control for 21 days. Faecal output was greater and faecal excretion of bile acids was higher in rats fed the HPS diets with higher DS compared with control rats fed the PS diet. Apparent protein digestibility in rats fed the HPS diets with higher DS was lower than that in control rats fed the PS diet. The pool size of caecal organic acids was not affected by diet. Apparent protein digestibility, faecal output and faecal bile acids excretion were significantly correlated with DS ($r = -0.994$, $p = 0.0059$; $r = 0.976$, $p = 0.0236$; and $r = 0.899$, $p = 0.0077$, respectively). Plasma cholesterol concentration was significantly lower in rats fed the HPS diets than in control rats fed the PS diet. The HPS diets resulted

in higher proportions of propionic acid, lactic acid and succinic acid and a lower proportion of *n*-butyric acid compared to the PS diet.

4.2.6.5. Hydroxypropyl distarch phosphate (E 1442)

Rats

The effects of hydroxypropyl distarch phosphate (HDP) of three different degrees of cross-linking (degree of cross-linking not specified; DS = 0.012, 0.010 and 0.013) on concentration of plasma cholesterol, apparent digestibility of protein, faecal excretion of bile acids, faecal output and caecal pool of organic acids such as acetic, propionic, butyric, lactic and succinic acid were studied in rats (Ebihara et al., 1998). The degree of cross-linking (DC) of the hydroxypropyl distarch phosphate was as follows: HDP-A 0.046 < HDP-B 0.093 < HDP-C 0.232. Male Wistar rats ($n = 6$) were fed a fibre-free, purified diet containing either HDP or gelatinised PS (100 g/kg) as a control for 21 days. Faecal output and faecal excretion of bile acids were increased in rats fed the HDP diets compared to controls. Apparent protein digestibility in rats fed the HDP diets with higher DC was lower than that in controls. The pool size of caecal organic acids, expressed as micromoles per caecum, was not affected by diet. Apparent protein digestibility was significantly correlated with the degree of swelling power (DSP) ($r = 0.996$, $p = 0.0028$), which was inversely related to DC. The HDP diets did not affect plasma cholesterol concentration. The HDP diets resulted in higher proportions of acetic acid, lactic acid and succinic acid and a lower proportion of *n*-butyric acid compared to the PS diet.

The physiological effects of six different types of HDP from tapioca starch with two different degrees of substitution and three different degrees of cross-linking were investigated in rats (Kishida et al., 2001). Male Wistar rats ($n = 6$) were fed a fibre-free, purified diet containing either gelatinised unmodified tapioca starch (50 g/kg diet) as control or gelatinised chemically modified tapioca starch (HDP, 50 g/kg) for 21 days. HDP with two different degrees of substitution (DS = 0.05 and 0.23) and three different DC were used as chemically modified starch sources. The wet weight and moisture of faecal output of the rats fed HDP with higher DS were 100% and 20% greater than that in the control rats, respectively. The weights of caecal wall and caecal contents were also 30% and 50% higher in the rats fed HDP with higher DS than those in the control rats. The pH of the caecal contents was more acidic in the rats fed HDP with higher DS than that in the control rats. Faecal excretion of bile acids was 40% higher in the rats fed HDP with higher DS than in the control rats. The degree of cross-linking had little influence on these effects. Plasma cholesterol concentration was 16% lower in the rats fed HDP with higher DS and highest DC than in the control rats. The concentrations of liver lipids and plasma triglycerides and the caecal pool of organic acids were not affected by diet. The apparent absorptions of Ca and Mg were not affected by diet, but those of Zn and Fe were 75% and 70% lower in the rats fed HDP with higher DS than in the control rats.

Male weanling rats (Holtzman strain; not further specified) were fed semipurified diets containing 15% or 35% of either hydroxypropyl distarch phosphate or unmodified starch for 28 days (Bruns and Hood, 1973). At the 35% level, mean weight gains, food consumption and protein energy ratio (PER) ($3.00 \pm 0.13/2.74 \pm 0.42$, modified/unmodified) were similar. Diarrhoea, caecal enlargement and depression of caecal pH from 7.2 to 5.0 were observed in the animals fed the modified starch. Aerobic microorganisms were 10- to 1,000-fold greater in the faeces from animals on the modified starch diet than those on unmodified starch. The changes were most pronounced in animals fed the 35% diet. After 180 days, all streptococci disappeared from the caecal microflora; coliforms declined from 10^7 to 10^4 /g faeces, while lactobacilli remained constant.

4.2.6.6. Starch sodium octenyl succinate (E 1450)

Special studies in young animals

Dogs

Pups from five Beagle dams (four of each sex per litter, between 5 and 9 days of age, and each dose group fed by a single dam) were administered 0 (water control), 5,000 or 10,000 mg/kg bw per day of OSA-modified starch or 5,000 or 10,000 mg/kg bw per day of a control starch for 6 weeks (RLMD, 1990; cited in JECFA, 2015). The starches were suspended in water (30%) and administered via gavage twice daily. Dams and pups had access to water and dog feed at all times. Pups were monitored for body weight, physical appearance, behaviour, unusual signs, haematology, blood chemistry, gross lesions and histopathological findings. A urine sample was collected from the bladder of each animal at

necropsy. There were no significant differences in blood chemistry, haematology or urine parameters among groups. No deaths, gross lesions or histological findings were attributable to the treatment.

Pigs

JECFA (2015) reported that 'the safety and effect of OSA-modified starch on the growth of piglets were investigated in a GLP-compliant 3-week toxicity study (Mahadevan et al., 2014). Two-day-old domestic Yorkshire cross-bred piglets (six of each sex per group; weighing 1.7–2.6 kg) were administered 500 mL/kg bw per day of milk containing 0, 2, 4 or 20 g OSA-modified starch per litre (equivalent to 0, 1,000, 2,000 or 10,000 mg/kg bw per day) for 3 weeks. The control, low-dose, mid-dose and high-dose groups also received amioca powder (control article) at levels of 8,000, 7,200, 6,400 or 0 mg/kg bw per day, respectively, to ensure that the total caloric intake was similar among groups, accounting for the decreased digestibility of OSA-modified starch. All animals were offered the test materials at a dose volume of 500 mL/kg bw per day via a feeding device, 6 times per day (~ 83.33 mL/kg bw per dose, 3 ± 0.25 h between doses). Administration of the test and control articles began on lactation day 2. A complete physical examination was conducted on all animals on day 4'.

All animals survived to scheduled necropsy on day 21, and there were no compound-related changes in clinical observations during the study. The test article was well tolerated by the piglets. No significant effects on haematology, clinical chemistry, organ weights or histopathological examination were observed. The authors concluded that administration of OSA-modified starch in the diet for a 3-week period after birth was well tolerated in piglets and that exposure to OSA-modified starch did not produce any definitive compound-related effects on growth or the clinical pathology parameters evaluated. Moreover, no effects attributable to the test article were observed upon macroscopic or microscopic evaluation. The NOAEL in this study was 10,000 mg/kg bw per day, the highest dose tested.

Observations in humans

JECFA (2015) reported that 'in a double-blind crossover study conducted to investigate the glycaemic response to OSA-modified starch, 30 healthy non-diabetic adult subjects (12 men and 18 women; mean age of 43 ± 3 years, age range 20–74 years) ingested 25 g of glucose or 25 g of OSA-modified starch after an overnight fast (Wolf et al., 2001). Blood samples were obtained at baseline and 15, 30, 45, 60, 90 and 120 min post-prandial for glucose analysis. There were no significant differences in mean fasting blood glucose concentrations between treatments. Mean peak incremental change from baseline and net incremental area under the curve were significantly lower in the OSA-modified starch group compared with the glucose group. Compared with the glucose treatment group, the post-prandial incremental change from baseline in blood glucose was significantly lower in the OSA-modified starch treatment group at 15 and 30 min and significantly higher at 120 min. There were no clinically significant differences in gastrointestinal symptoms observed between treatments, nor were there any adverse events reported in any subject. The authors concluded that OSA-modified starch was well tolerated by fasting healthy adults and attenuated the post-prandial glycaemic response compared with glucose (Wolf et al., 2001)'.

Studies in infants

According to the JECFA report (2015) 'the growth, acceptance and tolerance of female term infants fed either a milk-based formula containing OSA-modified starch (OSA-modified starch content in the range of 1.33–1.47 g/100 mL) or a milk-based formula containing distarch phosphate-modified tapioca starch (control) were examined in a randomised, multicentre, double-blind clinical study (MJNR, 1994; cited in JECFA, 2015). The starch content of both test formulas was the same as that in a marketed infant formula, on a weight-to-weight basis. One hundred and seven infants (55 in the OSA-modified starch group and 52 in the non-OSA-modified starch group) between 2 and 16 days of age were enrolled in the study. All subjects were provided the study formulas as the sole source of nutrition for 120 days'.

No significant differences in discontinuation rates were observed between treatment groups. There were no significant differences in weight gain at 30, 60 or 90 days observed among the groups. Furthermore, at 90 days of age, the intake of formula in the OSA-modified starch group was significantly higher than that in the control group, with a mean intake of 1,114 mL for the OSA-modified starch group and 947 mL for the non-OSA-modified starch group, although the authors noted that formula intake was not accurately determined for some of the subjects. No significant differences in growth, product assessment (satisfaction based on parental and infant criteria, including 'spit-up and

stool odour'), reported illnesses or 'symptoms of concern', or parental concerns were reported between groups' (MJNR, 1994; cited in JECFA, 2015).

The tolerability of formulas containing OSA-modified starch was further examined in a randomised, multicentre, double-blind, good clinical practice (GCP)-compliant trial (Borschel and Kajzer, 2011; cited in JECFA, 2015). Healthy term infants were fed either a commercial control formula or one of two experimental casein hydrolysate formula powders (EF-1 or EF-2). EF-1 was a casein hydrolysate-based infant formula containing iron, DE1 maltodextrin, DE15 maltodextrin, sucrose and OSA-modified starch (< 2%, not further specified). EF-2 was a casein hydrolysate-based infant formula containing OSA-modified starch (< 2%, not further specified), DE15 maltodextrin and sucrose. All formulas were provided *ad libitum*. One hundred and sixty-eight infants were enrolled from day 0 (birth) to day 8 of life and were followed until day 28 of life. Of these, 131 completed the study. Randomisation was achieved for sex, ethnicity, race, age, birth weight and length.

No statistically significant differences were reported for weight, length and their respective gains, as well as dropout rates due to intolerance. No statistically significant differences were observed in mean rank stool consistency, percentage of watery stools, percentage of stools of other consistencies, percentage of stool colours, predominant stool consistency or colour, percentage of feedings with spit-up and/or vomit associated with feeding, and average daily study product intake. Infants provided the EF-1 exhibited a statistically significant increase in the number of stools compared with those provided EF-2. Parents of infants fed the control formula responded more favourably when ranking the formula odour in the Formula Satisfaction Questionnaire. Parents of infants fed EF-1 reported more gassy responses in the Infant Feeding & Stool Patterns Questionnaire when compared with parents of infants fed the control formula or EF-2.

The majority of the adverse events reported were either mild or moderate, with gastrointestinal disorders being the most frequently reported adverse event. The number of adverse vomiting events was significantly greater in the EF-1 treatment group than in controls. For most variables, tolerance of all three formulas was similar, although a significantly greater number of stools occurred in the EF-1 group; this was thought to be related to the absence of the stabiliser in this formula. The authors noted that there were no clinically relevant differences in serious adverse events between the treatment groups, and concluded that, overall, no safety concerns were noted with the experimental formulas, indicating a lack of concern regarding the inclusion of OSA-modified starch (Borschel and Kajzer, 2011; cited in JECFA, 2015).

A number of additional infant growth studies have been undertaken using a proprietary infant formula containing OSA-modified starch as a control formula for alternative experimental specialised formulas. These studies included those conducted by Burks et al. (2008), Scalabrin et al. (2009) and Borschel et al. (2013).

A similar growth study was conducted in healthy term infants fed an amino acid-based formula or an extensively hydrolysed casein-based formula containing 1.6% OSA-modified starch (Borschel et al., 2013). This was a randomised, double-blind, parallel group design study, in which 213 infants were enrolled between 0 and 9 days of age and studied until 112 days of age. The formulas were designed to be the sole source of nutrition throughout the study. The primary outcome variable was weight gain between 14 and 112 days of age, whereas secondary measures included length, head circumference, formula intake, daily stool number, mean rank stool consistency and serum albumin. The dose of formula was similar across the groups, and appropriate parameters were measured and noted at specific time intervals during the study. The dose of OSA-modified starch was calculated to be 2.5 g/kg bw per day. A total of 134 infants completed the study, with similar demographic characteristics between groups.

Formula intakes recorded among the groups were similar, as were the numbers of infants who finished the study early because of intolerance symptoms. There were no statistically significant differences between groups in weight, length, head circumference or mean serum albumin concentration. There were significant differences in stool patterns, with the group receiving OSA-modified starch having a significantly greater number of daily stools and average mean rank stool consistency at 14 and 28 days of age, which were considered to be due to this formula containing palm olein oil as a source of fat.

According to JECFA (2015), 'OSA-modified starch is also currently being marketed on an international basis within a nutritionally complete, hypoallergenic formula containing hydrolysed protein with free amino acids for infants with food allergies, sensitivity to intact protein or protein maldigestion. Distribution of the formula containing 2% OSA-modified starch commenced in November 2012 in a number of countries located in Central and South America, Asia Pacific and the United

Kingdom. Patient exposure is still fairly limited, as only 167,424 patient treatment days (a patient treatment day is defined as 0.8 L of prepared formula) had been distributed as of 30 October 2013. The adverse event reports received have been primarily related to gastrointestinal symptoms that are within the expected safety profile for this product when fed to the intended population, according to directions provided on the label or as instructed by a health-care professional. To date, the use of OSA-modified starch has apparently been well tolerated when administered to infants through its intentional use in a specialised infant formula' (ISDI, 2013; cited in JECFA, 2015).

Overall, the Panel concluded that OSA-modified starch up to a single dose of 25 g (25,000 mg/person) was well tolerated by fasting healthy adults. However, the Panel noted reports on gastrointestinal symptoms from the post-marketing surveillance study conducted in infants with hypoallergenic formula containing 2% of OSA-modified starch (about 24,000 mg/person) (EFSA CONTAM Panel, 2012b; DH, 2013).

4.3. Discussion

The present opinion deals with the re-evaluation of the safety of modified starches comprising oxidised starch (E 1404), monostarch phosphate (E 1410), distarch phosphate (E 1412), phosphorylated 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) when used as food additives. These modified starches are authorised food additives in the EU according to Annex II and Annex III to Regulation (EC) No 1333/2008.

Starch typically consists of two polymers of glucose exhibiting a variable proportion: amylose with an almost linear structure, and amylopectin, which is highly branched. In amylose, the glucose monomers (pyranosic form) are linked by α -1,4-glycosidic links, while amylopectin contains additionally α -1,6-glycosidic bonds. Starches for commercial use are generally produced from potatoes, cereals or other sources. Indicatively, polymer molecular weights fall in the following ranges (rounding-off to one figure): for amylopectins, from 50×10^6 Da to 500×10^6 Da (higher values have been reported by Yoo and Jane, 2002), with an average near 100×10^6 Da; for amyloses, from 2×10^3 to $4,000 \times 10^3$ Da.

The most common chemical modification of the so-called 'native' starches includes oxidation, esterification and etherification. In the present opinion, modified starches have been identified with CAS Registry numbers and, when available, EC numbers that were subject to confirmatory steps to minimise the uncertainty of an equivocal identification met in few cases. In modified starches, the chemical and physical characteristics of the native substances are altered in order to improve the functional properties for particular food applications. In general, the extent of modification required to distinctly alter the functional characteristics of native starches is low, as imposed by Commission Regulation (EU) No 231/2012.

The Panel noted that, according to the EU specifications, of the toxic elements arsenic, lead and mercury are accepted up to concentrations of 1, 2 and 0.1 mg/kg, respectively. The Panel considered that contamination at such levels could have a significant impact on exposure to these metals, for which exposure is already close to the health-based guidance values benchmark doses (lower confidence limits) established by EFSA.

Several data on enzymatic degradation of modified starches are available using pancreatin, saliva or amylase. In comparison with unmodified starches, the digestibility of the modified starches was slightly reduced or showed no difference. Despite the absence of ADME data for two modified starches (E 1451 and E 1452) and the absence of *in vivo* studies in humans for some other modified starches, the Panel considered the ADME database sufficient to conclude that modified starches would not be absorbed intact but significantly hydrolysed by intestinal enzymes and then fermented by intestinal microbiota in humans to SCFA such as acetic, propionic and butyric acids, which are absorbed from the colon, and considered of no safety concern by the Panel.

In the case of starch aluminium octenyl succinate (E 1452) and considering the intestinal hydrolysis and fermentation of starches, the Panel noted that the biological fate of aluminium from this modified starch is not documented.

Acute oral toxicity data were available only for distarch phosphate (E 1412) in several species. LD₅₀ values were all above 7,000 mg/kg bw.

Short-term and/or sub-chronic (90-day) studies in rats were available for all modified starches, except monostarch phosphate (E 1410). Occasionally, also studies in dogs, pigs or hamsters were

available. The modified starches were given at dietary levels up to 70%. The test duration was up to 90 days. Effects on body weight and feed consumption were not observed up to dietary levels of 25%. Caeca weights were increased at exposure levels of 30% and higher, but histopathological changes were not observed. The only significant histopathological change was the presence of pelvic and/or corticomedullary mineralisation in the kidneys, which was observed upon administration of modified as well as unmodified starches and occurred more pronounced in females than in males.

In a 90-day study with acetylated oxidised starch (E 1451) in rats, a NOAEL of 10% in the diet (equal to 5,900 mg/kg bw per day) was determined, based on hyperplasia of the transitional epithelium of the urinary bladder and the kidneys, which were observed at 18,000 mg/kg bw per day, the next higher dose in this study.

Evaluation of genotoxicity of the modified starches evaluated in the present opinion was performed *in silico*, since no genotoxicity studies were available. The Panel concluded that the *in silico* analysis of the substructures of modified starch moieties did not identify any relevant alert for genotoxicity, and concluded that modified starches do not raise concern for genotoxicity.

Two chronic (52-week) studies in rats were available, one with acetylated distarch phosphate (E 1414) and one with acetylated distarch adipate (E 1422). At necropsy, relative organ weights showed no differences among the groups, except for caecal enlargement. Histopathological examination of kidneys demonstrated the presence of treatment-related pelvic nephrocalcinosis. A clear correlation was observed between the increased incidence of pelvic nephrocalcinosis, increased accumulation of calcium in the kidneys and increased urinary excretion of calcium.

A carcinogenicity study (89-week) in mice was available for hydroxypropyl distarch phosphate (E 1442). There was no evidence of carcinogenicity. This study demonstrated some histopathological changes in the kidneys of mice, characterised by intratubular mineralisation, which according to the authors were of no toxicological significance for human health. The Panel agreed with this conclusion.

Carcinogenicity studies in rats were available for phosphated distarch phosphate (E 1413), acetylated distarch phosphate (E 1414), acetylated starch (E 1420), acetylated distarch adipate (E 1422), hydroxypropyl distarch phosphate (E 1442) and starch sodium octenyl succinate (E 1450). There was no evidence of carcinogenicity. These studies in rats did not reveal any significant effect, except for caecal enlargement. As the effect on the caecum observed was not associated with histopathological changes, it was considered to be of no toxicological significance for humans.

Kidney lesions (pelvic and corticomedullary mineralisation) developed in rats fed high levels (up to 62%; 31,000 mg/kg bw per day) of most of the modified starches evaluated, except starch sodium octenyl succinate (E 1450). The lesions were considered to be associated with an imbalance of Ca/P and Mg in the diet (Documentation provided to EFSA n. 28; Hodgkinson et al., 1982). The Panel noted that studies in the 1970s and 1980s demonstrated a credible mechanistic explanation for the nephrocalcinosis in rats and mice related to effects on Ca/P balance (Ritskes-Hoitinga et al., 1989, 1991, 1992; EFSA, 2007b).

Reproductive toxicity studies in rats were available for phosphate distarch phosphate (E 1413), acetylated distarch phosphate (E 1414), acetylated starch (E 1420) and acetylated distarch adipate (E 1422). No effects on reproductive performance and no maternal and developmental effects were observed in the three-generation reproductive toxicity studies at dietary levels of up to 62% (equivalent to 31,000 mg/kg bw per day). No prenatal developmental toxicity studies were available.

The Panel noted that starch sodium octenyl succinate (E 1450) up to a single dose of 25,000 mg was well tolerated by fasting healthy adults. However, the Panel noted reports on gastrointestinal symptoms in a post-marketing surveillance study (ISDI, 2013; cited in JECFA, 2015) conducted in infants with hypoallergenic formula containing 2% of OSA-modified starch (24,000 mg/person).

The Panel noted that it may be considered to establish specific purity criteria for the use of modified starches in food for infants and young children (FC 13.1).

In studies with phosphated distarch phosphate (E 1413), acetylated distarch phosphate (E 1414) and acetylated starch (E 1420) in healthy human volunteers, no adverse effects were reported at doses of 60,000 mg/person.

To assess the dietary exposure to modified starches (E 1404–E 1451) from their use as food additives, the combined exposure was calculated based on (1) maximum reported use levels provided to EFSA (defined as the *maximum level exposure assessment scenario*) and, (2) reported use levels (defined as the *refined exposure assessment scenario, brand-loyal and non-brand-loyal scenario*).

Modified starches are authorised in a wide range of foods. The Panel did identify brand loyalty to specific food categories in infants and toddlers (e.g. processed cereal baby foods, unflavoured fermented milk products and flavoured fermented milk products). Further, the Panel considered that

the non-brand-loyal scenario covering other population groups was appropriate and realistic scenario for risk characterisation because it is assumed that the population would probably be exposed long-term to the food additive present at the mean reported use level in processed food.

A refined estimated exposure assessment scenario taking into account the FSMP for infants and young children (FC 13.1.5.1 Dietary foods for infants for special medical purposes and special formulae for infants and FC 13.1.5.2 Dietary foods for babies and young children for special medical purposes as defined by Commission Directive 1999/22/EC) was also performed to estimate exposure of infants and toddlers who may be on a specific diet. Considering that this diet is required due to specific needs, it is assumed that consumers are loyal to the food brand, therefore the refined brand-loyal exposure assessment scenario was performed.

A specific *food supplement consumers only* scenario was also performed to estimate exposure of children, adolescents, adults and the elderly, as exposure via food supplements may deviate largely from that via food, and the number of food supplement consumers may be low depending on populations and surveys.

The refined estimates were based on 36 out of 72 food categories in which modified starches are authorised. The Panel considered that the uncertainties identified would, in general, result in an overestimation of the exposure to modified starches as a food additive in European countries for the maximum level exposure scenario. However, the Panel noted that given the information from the Mintel's GNPD, it may be assumed that modified starches are used in food categories ($n = 13$) for which no data have been provided by food industry. The main food categories, in terms of amount consumed, not taken into account were processed fish and fishery products, including molluscs and crustaceans, breakfast cereals, salads and savoury-based sandwich spreads. According to the Mintel GNPD, in the EU market, these categories are labelled with modified starches. Therefore, the Panel considered that if these uncertainties were confirmed, it would therefore result in an underestimation of the exposure.

The Panel further noted that the exposure to modified starches (E 1404–E 1451) from their use according to Annex III to Regulation (EC) No 1333/2008 (Parts 1, 3 and 5) was not considered in the exposure assessment.

Separate scenarios were carried out for the exposure assessment of starch aluminium octenyl succinate (E 1452), taking into account the consumption of food supplements for consumers only and based on the MPL (*regulatory maximum exposure assessment* scenario) and on the maximum reported use level (*maximum reported level exposure assessment* scenario). Exposure to aluminium from the use of E 1452 as a food additive was also estimated.

Exposure to aluminium from the use of starch aluminium octenyl succinate (E 1452) in the *regulatory maximum level exposure assessment* scenario ranged for all population groups from 0.8% to 26% of the TWI of 1 mg aluminium/kg bw established by EFSA (2008) at the mean, and up to 47% at the 95th percentile. For the *maximum reported level exposure assessment* scenario, based on the usage levels provided by food industry, exposure to aluminium from E 1452 ranged from < 0.1 at the mean, up to 2.5% for the 95th percentile, across population groups. Furthermore, according to the information provided by industry, the content of aluminium in E 1452 is significantly lower than the limit set in the EU specifications for E 1452.

The Panel also noted that the refined exposure estimates are based on information provided on the reported levels of use of modified starches. If actual practice changes, this refined estimates may no longer be representative and should be updated.

5. Conclusions

General population

Following the conceptual framework for the risk assessment of certain food additives re-evaluated under Commission Regulation (EU) No 257/2010 (EFSA ANS Panel, 2014) and given that:

- adequate combined exposure data were available; in the general population, the 95th percentile of the refined exposure, calculated based on the use levels reported from food industry, was up to 3,053 mg/kg bw per day for toddlers (brand-loyal consumer scenario);
- an indicative refined exposure to modified starches (E 1404–E 1451) of up to 991 mg/kg bw per day has been calculated at the 95th percentile for children, for the population consuming food supplements;
- exposure to starch aluminium octenyl succinate (E 1452) for food supplement consumers only at the 95th percentile was 22.1 mg/kg bw per day (*regulatory maximum level exposure*

assessment scenario) and 1.2 mg/kg bw per day (maximum reported level exposure scenario) in the elderly;

- their structural, physicochemical and biological similarities, allow for read-across between all the modified starches;
- the ADME database is sufficient to conclude that, in humans, modified starches would not be absorbed intact, but significantly hydrolysed by intestinal enzymes and then fermented by the intestinal microbiota,
- using the read-across approach, adequate data on short- and long-term toxicity and carcinogenicity and reproductive toxicity are available,
- no treatment-related effects relevant for human risk assessment were observed in long-term studies in rats fed very high levels of modified starches (up to 31,000 mg/kg bw per day);
- although no genotoxicity data on the modified starches evaluated in the present opinion were available, modified starches are not of genotoxic concern based on *in silico* analysis,
- modified starches (i.e. E 1413, E 1414, E 1420, E 1450) were well tolerated in adults up to a single daily dose of 60,000 mg/person (860 mg/kg bw),

the Panel concluded that there is no safety concern for the use of modified starches as food additives at the reported uses and use levels and that there is no need for a numerical ADI.

Infants and young children consuming foods for special medical purposes and special formulae

Concerning the use of starch sodium octenyl succinate (E 1450) in 'dietary foods for special medical purposes and special formulae for infants' (food category 13.1.5.1) and of E 1404, E 1410, E 1412, E 1413, E 1414, E 1420, E 1450 and E 1451 in food belonging to food category 13.1.5.2 and given that:

- for populations consuming foods for special medical purposes and special formulae, the 95th percentile of exposure calculated based on the maximum use levels reported from food industry was up to 5,286 mg/kg bw per day for infants;
- infants and young children consuming foods belonging to these food categories may show a higher susceptibility to the gastrointestinal effects of modified starches than their healthy counterparts due to their underlying medical condition;
- no effects on body weight and food intake were observed in male and female neonatal pigs exposed to 10,000 mg/kg bw per day of OSA-modified starch (E 1450) in formula for 21 days;
- OSA-modified starch (E 1450), up to a single dose of 25,000 mg/person, was well tolerated by fasting healthy adults, but gastrointestinal symptoms were reported in infants with hypoallergenic formula containing 2% of OSA-modified starch (about 24,000 mg/person);
- available information on the clinical studies in infants is limited and results refer to the feeding of formula containing OSA-modified starch in concentrations below 2%, the current authorised MPL,

the Panel concluded, that the available data do not allow for an adequate assessment of the safety of the use of starch sodium octenyl succinate (E 1450) in 'dietary foods for special medical purposes and special formulae for infants' (food category 13.1.5.1) or of E 1404, E 1410, E 1412, E 1413, E 1414, E 1420, E 1450 and E 1451 in foods belonging to food category 13.1.5.2, in infants and young children consuming these foods at the presently authorised maximum use levels of 20,000 or 50,000 mg/kg, respectively.

6. Recommendations

The Panel recommended that:

- the European Commission considers revising the maximum limits for the toxic elements arsenic, lead and mercury present as impurities in the EU specifications for all modified starches re-evaluated in the present opinion (E 1404, E 1410, E 1412, E 1413, E 1414, E 1420, E 1422, E 1440, E 1442, E 1450, E 1451 and E 1452) to ensure that these food additives will not be a significant source of exposure to these toxic elements in food;
- the European Commission considers revising specifications, including harmonisation of microbiological criteria for polysaccharides such as modified starches and gums, and taking into account future availability of specific methods of analysis of modified starches;

- the European Commission seeks confirmation on the actual use of starch aluminium octenyl succinate (E 1452) in its currently permitted use limited to food supplements (only vitamin preparations for encapsulation purposes)
- additional data should be generated to assess the potential health effects of starch sodium octenyl succinate (E 1450) when used in 'dietary foods for special medical purposes and special formulae for infants' (food category 13.1.5.1) or of E 1404, E 1410, E 1412, E 1413, E 1414, E 1420, E 1450 and E 1451 in foods belonging to food category 13.1.5.2
- due to the discrepancies observed between the data reported from industry and the Mintel database, where modified starches (E 1404–E 1451) are labelled in more products than in food categories for which data were reported from industry, the Panel recommended collection of data on usage and use levels of modified starches (E 1404–E 1451) in order to perform a more realistic exposure assessment.

Documentation provided to EFSA

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Glossary and/or Abbreviation

AAF	Association des Amidonniers et Féculiers (European Starch Industry Association)
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, excretion
AESGP	Association of the European Self-Medication Industry
AFC Panel	EFSA former Panel on Food Additives, Flavourings, Processing Aids and Food Contact Materials

ANS Panel	EFSA Panel on Food Additives and Nutrient Sources added to Food
BOD	biochemical oxygen demand
BUN	blood urea nitrogen
bw	body weight
CAS	Chemical Abstract Service
CCCCF	Codex Committee on Contaminants in Foods
CONTAM Panel	EFSA Panel on Contaminants in the Food Chain
DC	degree of cross-linking
DS	degree of substitution
DSP	degree of swelling power
EC	Enzyme Commission.
ECHA	European Chemicals Agency
EDA	European Dairy Association
EMA	European Medicines Agency
FASEB	Federation of American Societies for Experimental Biology
FAO/WHO	Food and Drug Organisation/World Health Organisation
FCS	food categorisation system
FDA	Food and Drug Administration
FDE	FoodDrinkEurope
FSE	Food Supplements Europe
FT-IR	Fourier transform IR spectroscopy
GC–MS	gas chromatography–mass spectrometry
GCP	good clinical practice
GHP	good hygiene practices
GLP	good laboratory practice
GMP	good manufacturing practices
GNPD	Mintel's Global New Products Database
GRAS	generally recognised as safe
HACCP	hazard analysis critical control point
HAMS	high-amylose maize starch
HAMSA	high-amylose maize starch, acetylated
HAMSB	high-amylose maize starch, butyrylated
HAMSP	high-amylose maize starch, propionylated
HDP	hydroxypropyl distarch phosphate
HPS	hydroxypropyl starches
JECFA	Joint FAO/WHO Expert Committee on Food Additives
ICGA	International Chewing Gum Association
IR	infrared
ISS	Istituto Superiore di Sanità
LAMS	low-amylose maize starch
LD ₅₀	lethal dose, median
ML	maximum level
MPL	maximum permitted levels
MS	mass spectrometry
NDA Panel	EFSA Panel on Dietetic Products, Nutrition and Allergies
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
OECD	Organisation for Economic Co-operation and Development
OSA	octenyl succinic anhydride
PER	protein energy ratio
PMR	proton magnetic resonance
PN	pelvic nephrocalcinosis
PS	potato starch
PTWI	provisional tolerable weekly intake
QS	<i>quantum satis</i>
QSAR	quantitative structure–activity relationships
RBC	red blood cells

ROS	reactive oxygen species
RS	resistant starch
RSD	relative standard deviation
SCF	Scientific Committee on Food
SCFA	short-chain fatty acids
SGOT	serum glutamic-oxaloacetic transaminase
SGPT	serum glutamic-pyruvic transaminase
SNE	Specialised Nutrition Europe
SSOS	starch sodium octenyl succinate
TAMC	total anaerobic microbial count
TG	test guideline
TYMC	total combined yeast and mould count
TWI	tolerable weekly intake
WBC	white blood cells
WG	Working Group

Appendix A – Summary of reported use levels (mg/kg or mg/L as appropriate) of modified starches (E 1404–1452) provided by industry

Appendix B – Number and percentage of food products labelled with modified starches (E 1404–1452) out of the total number of food products present in the Mintel GNPD per food subcategory between 2011 and 2016

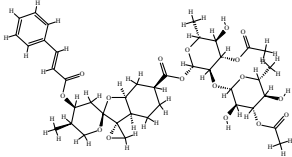
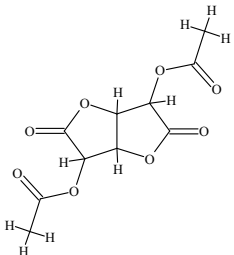
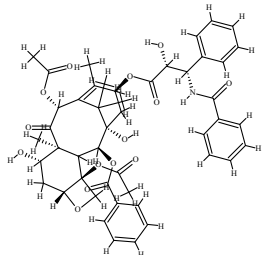
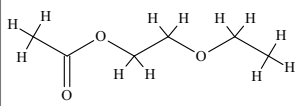
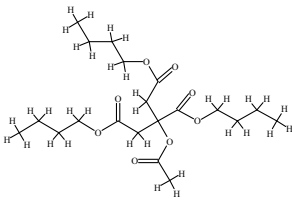
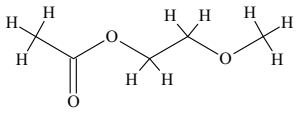
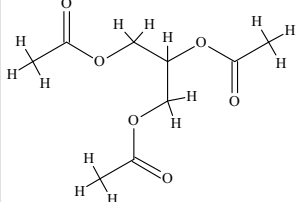
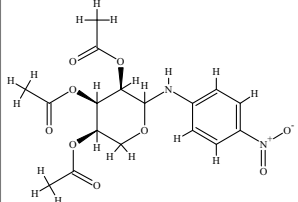
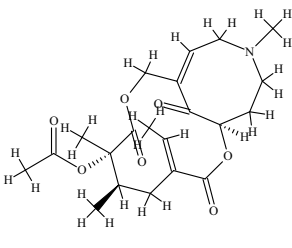
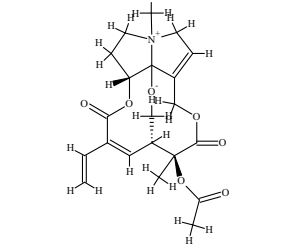
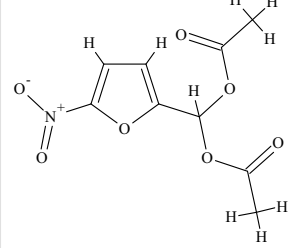
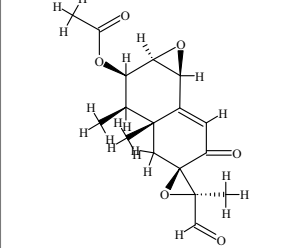
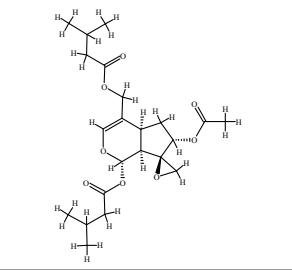
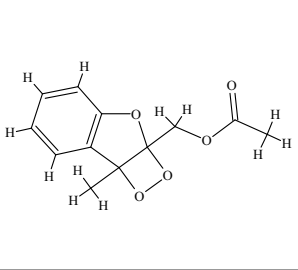
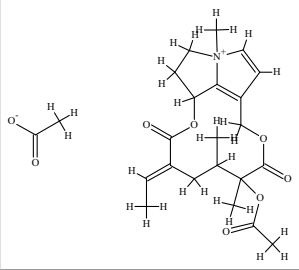
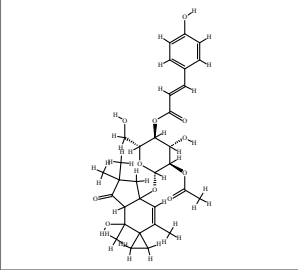
Appendix C – Concentration levels of modified starches (E 1404–1452) used in the exposure assessment scenarios (mg/kg or mL/kg as appropriate)

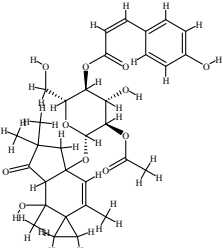
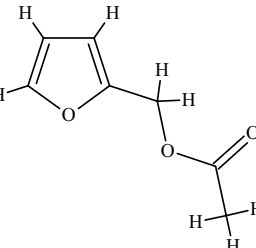
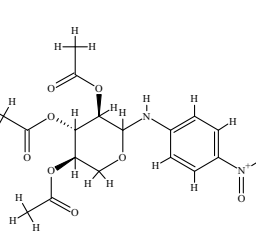
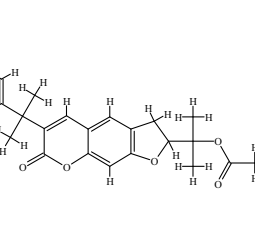
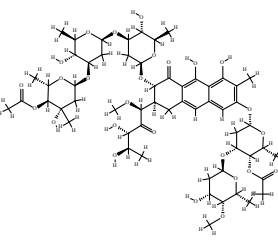
Appendix D – Summary of total estimated exposure to modified starches from their use as food additives for the maximum level exposure scenario and the refined exposure assessment scenarios per population group and survey: mean and 95th percentile (mg/kg bw per day)

Appendix E – Main food categories contributing to exposure to modified starches using the maximum level exposure assessment scenario and the refined exposure assessment scenarios (> 5% to the total mean exposure)

Appendices A–E can be found in the online version of this output ('Supporting information' section): <https://doi.org/10.2903/j.efsa.2017.4911>

Appendix F – Substances containing the alerting acetate ester group present in the ISSSTY database

1 (ID:1897) 	2 (ID:3942) 	3 (ID:3217) 	4 (ID:1833) 
OVERALL: 1 YES CAS: 63166-73-4	OVERALL: 1 NO CAS: 642-83-1	OVERALL: 1 NO CAS: 33069-62-4	OVERALL: 1 NO CAS: 111-15-9
5 (ID:1243) 	6 (ID:1228) 	7 (ID:1092) 	8 (ID:6909) 
OVERALL: 1 NO CAS: 77-90-7	OVERALL: 1 NO CAS: 110-49-6	OVERALL: 1 NO CAS: 102-76-1	OVERALL: 3 YES CAS: 109527-10-8
9 (ID:5582) 	10 (ID:5580) 	11 (ID:3830) 	12 (ID:1505) 
OVERALL: 3 YES CAS: 60872-63-1	OVERALL: 3 YES CAS: 33979-15-6	OVERALL: 3 YES CAS: 92-55-7	OVERALL: 3 YES CAS: 56299-00-4
13 (ID:1088) 	14 (ID:7204) 	15 (ID:6322) 	16 (ID:5828) 
OVERALL: 3 YES CAS: 18296-45-2	OVERALL: 3 NO CAS: 128753-96-8	OVERALL: 3 NO CAS: 71673-83-1	OVERALL: 3 NO CAS: 125761-28-6

17 (ID:5825) 	18 (ID:2045) 	19 (ID:6910) 	20 (ID:6195) 
OVERALL: 3 NO CAS: 125676-80-4	OVERALL: 3 NO CAS: 623-17-6	OVERALL: Inc YES CAS: 109785-47-9	OVERALL: Inc YES CAS: 14882-94-1
21 (ID:5507)  OVERALL: Inc NO CAS: 7059-24-7			

The field 'OVERALL' refers to the overall outcome of the Ames test: Negative = 1; Positive = 3; Inconclusive = Inc.

YES/NO labels refer to the presence of other structural alerts, different from the 'specific acetate esters' alert.

Evaluation of certain food additives

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



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Evaluation of certain food additives

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



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Organization of the
United Nations



World Health
Organization

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Committee on Food Additives

(WHO technical report series; no. 1014)

ISBN 978-92-4-121023-2

ISSN 0512-3054

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Printed in Malta.

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Food Additives
Geneva, 12–21 June 2018

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¹ Unable to attend the meeting.

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 β -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 GSFA, including further alignments of food additive provisions in the Codex commodity standards with the corresponding provisions of the GSFA.

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

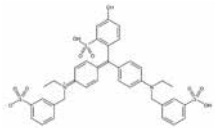
2.3.1 Corrigenda for specifications monographs

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^a

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)

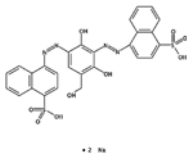
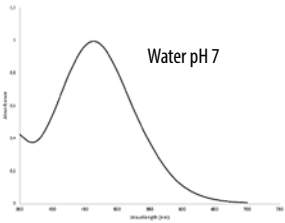
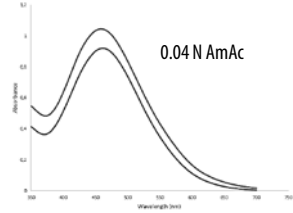
Food additive	Original text	New text	Additional information
Monograph 20 (2017)	Pyrogenic silica is produced in an essentially anhydrous state, whereas the wet process products are obtained as hydrates or contain surface absorbed water.	Pyrogenic silica is produced in an essentially anhydrous state, 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

Residual solvents (Vol. 4)	Acetone:	Not more than 30 mg/kg
	Hexane:	Not more than 25 mg/kg
	Methanol:	}
	Ethanol:	Not more than 50 mg/kg
	Isopropanol:	
	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, 82nd meeting, 2016)

JECFA name	Sample weight	Structure	Spectral data	Visible absorption spectrum
Brown HT	245.6 mg		Water, pH 7 λ_{max} = 464 $A = 0.9957$ Spec abs = 403 $a = 40.3$ Water λ_{max} = 464 $A = 0.9804$ Spec abs = 397 $a = 39.7$ 0.04 N AmAc λ_{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 λ_{max} = 461 $A = 0.9206$ Lake (red) 0.04 N AmAc λ_{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/m³) 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 γ -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 (CIFOCCoss) 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 ^{a,b}				Total methacrylic acid exposure (µg/kg bw per day) ^c
	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
^a 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.
^b 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.
^c 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.

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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 (LD₅₀) 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/m³]). 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/m³, 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/m³) 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/m³) for 2 hours daily from gestation days 6 to 15. There were no treatment-related adverse effects. The NOAEL was 8315 mg/m³ [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 ^{a,b}			
		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 ^c	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 ^c	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

^a 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.

^b 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.

^c 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.

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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 factor² 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

² The previous Committee used the term “safety factor”.

($\leq 4\%$), fluorescein (≤ 20 mg/kg), inorganic iodides ($\leq 0.1\%$), lead (≤ 2 mg/kg) and zinc (≤ 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) ^a
Analytical levels ^b	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 ^c	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

^a The upper bound of the range is the maximum of the 90th and 95th percentiles.^b Studies from the Republic of Korea [18] and the USA [19, 20] for consumers only.^c From published national studies [21, 22]) and the Committee's assessment based on consumption data from CIFOCCs 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 CIFOCCs 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.

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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 (≤ 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 F₁ 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) ^a
National ^b	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 ^c	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

^a The upper bound of the range is the maximum of the 90th and 95th percentiles.

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

^c 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.

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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*)- β , ϵ -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*- β , ϵ -carotene-3,3'-diol) and lutein dipalmitate (helenien; β , ϵ -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*- β , β -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 (%) ^a	
	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

^a 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.

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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 Pharmacopeia [1] and United States Pharmacopeia 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}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/m³) 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 CIFOCoSS 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 ^{a,b}		
	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

^a 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.

^b 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.

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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-phycocyanin 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 F₀ 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). F₁ and F₂ offspring were not directly exposed to the test material. At 30% dried spirulina in the diet, reduced F₁ pup weight at birth and reduced F₁ survival rate on postnatal days 0–4 were reported; no effects were observed in the F₂ animals [26]. The Committee noted that there was no effect on pup survival rate in a 2-generation toxicity study in which F₀ 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 (%) ^a	
	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

^a 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.

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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 α,β -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 α,β -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 α,β -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 α,β -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 α,β -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 α,β -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^{a,b,c,d}

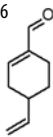
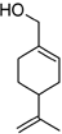
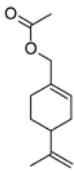
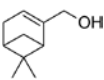
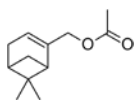
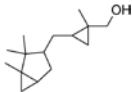
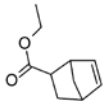
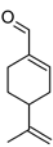
Flavouring agent	No.	CAS no. and structure	Step 4 ^e Does the highest dietary exposure estimate exceed the threshold of toxicological concern?	Conclusion based on current estimated dietary exposure
Structural class I				
Mixture of 1-vinyl-3-cyclohexenecarbaldehyde and 4-vinyl-1-cyclohexenecarbaldehyde	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
Structural class II				
(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
Structural class III				
(±)-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 ^e Does the highest dietary exposure estimate exceed the threshold of toxicological concern?	Conclusion based on current estimated dietary exposure
Flavouring agent excluded at Step 1 of the Procedure				
<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

^a 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.

^b 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.

^c 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.

^d 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.

^e 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.

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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 conditional³ 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

³ "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 µg/day for the four regions. For the other flavouring agents, the estimated daily dietary exposures range from 0.05 to 0.3 µg/day (MSDI values) and from 3 to 450 µg/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.

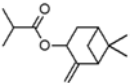
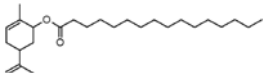
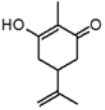
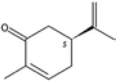
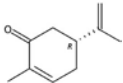
[Table 13](#) summarizes the evaluations of the three flavouring agents (Nos 2242–2244) in the group of carvone and structurally related substances.

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^{a,b}

Flavouring agent	No.	CAS no. and structure	Step 4 ^c Does the highest dietary exposure estimate exceed the threshold of toxicological concern?	Conclusion based on current estimated dietary exposure
Structural class I				
Pinocaryl isobutyrate	2242	929116-08-5 	No, SPET: 450	No safety concern
Carvyl palmitate	2243	929222-96-8 	No, SPET: 3	No safety concern
Structural class III				
6-Hydroxycarvone	2244	51200-86-3 	No, SPET: 4	No safety concern
Flavouring agents not evaluated according to the Procedure				
(+)-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

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

^b 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.

^c 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.

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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

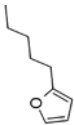
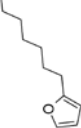
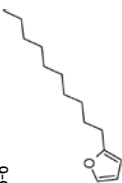
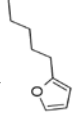
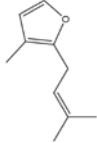
The Committee concluded that the 39 previously evaluated flavouring agents in this group of furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers that were re-evaluated at the present meeting (Nos 1491–1529 and 2103–2105) 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.

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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^{a,b,c,d}

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? ^e	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
Structural class III						
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

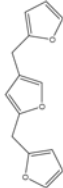
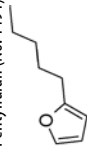
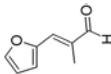
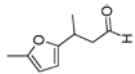
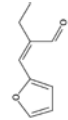
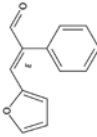
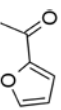
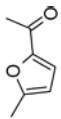
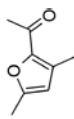
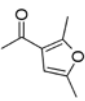
Step 4 Does the higher of the two predicted dietary exposure estimates exceed the threshold of toxicological concern value for the structural class?*			Step 5 Does a NOAEL exist for the flavouring agent or a structural relative that provides an adequate margin of exposure?		Structural relative name (No.) and Structure	Conclusion based on current estimated dietary exposure
Flavouring agent	No.	CAS no. and structure				
2,4-Difurfurylfuran	1496	64280-32-6 	Yes, SPET: 400	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.	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

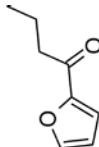
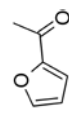
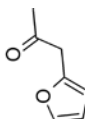
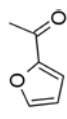
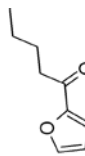
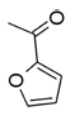
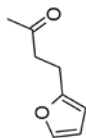
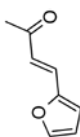
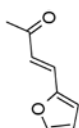
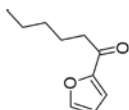
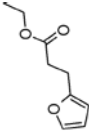
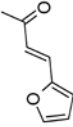
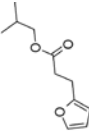
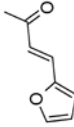
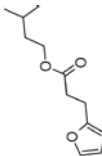
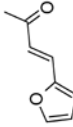
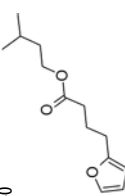
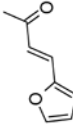
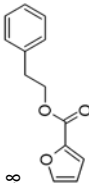
Step 4 Does the higher of the two predicted dietary exposure estimates exceed the threshold of toxicological concern value for the structural class?*				Step 5 Does a NOAEL exist for the flavouring agent or a structural relative that provides an adequate margin of exposure?	Structural relative name (No.) and Structure	Conclusion based on current estimated dietary exposure
Flavouring agent	No.	CAS no. and structure				
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	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
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.	4-(2-Furyl)-3-buten-2-one (No. 1511) 	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

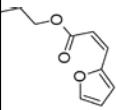
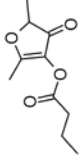
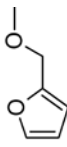
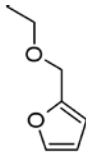
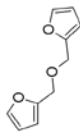
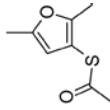
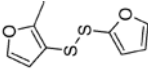
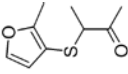
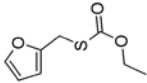
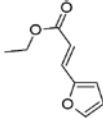
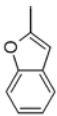
Step 4		Step 5		Conclusion based on current estimated dietary exposure
Flavouring agent	No.	Does the higher of the two predicted dietary exposure estimates exceed the threshold of toxicological concern value for the structural class?	Does a NOAEL exist for the flavouring agent or a structural relative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

^a 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.

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

^c Step 2: All 39 flavouring agents belong to structural class III.

^d 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.

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

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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 β -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.

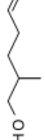
No adequate studies on the additional flavouring agents were available. For previously evaluated flavouring agents in this group, studies of acute toxicity (Nos 336, 1269, 1286 and 1640), studies of short-term toxicity (Nos 330, 332 and 333) and studies of genotoxicity (Nos 315, 329, 330, 333, 334, 346, 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^{a,b}

Flavouring agent	No.	CAS no. and structure	Step 4 ^c 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
Structural class I					
<i>trans</i> -6-Octenal	2240	63196-63-4 	No, SPET: 40	NR	No safety concern
2,6-Dimethyl-5-heptenol	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

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

^b Step 2: Two flavouring agents (Nos 2240–2241) are in structural class I.

^c 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.

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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.
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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 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 γ -pyrone. It is a hydroxyl-substituted 4H-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.

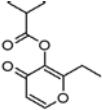
Step 4. The highest estimated dietary exposure for maltol (No. 1480) is above the threshold of toxicological concern (i.e. 540 $\mu\text{g/day}$ for class II). Accordingly, the evaluation of this flavouring agent proceeded to Step 5 of the Procedure.

The highest estimated dietary exposure for ethyl maltol isobutyrate (No. 2252) is above the threshold of toxicological concern (i.e. 90 $\mu\text{g/day}$ 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 $\mu\text{g/day}$ or 174 $\mu\text{g/kg bw per day}$) 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 $\mu\text{g/day}$ or 7 $\mu\text{g/kg bw per day}$) 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^{a,b}

Flavouring agent	No.	CAS no. and structure	Step 4: Does the highest dietary exposure estimate exceed the threshold of toxicological concern?	Step 5 ^d 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	Conclusion based on current estimated dietary exposure
Structural class I						
Maltol ^e	1480	118-71-8 	Yes, SPET: 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.	Ethyl maltol ^f (No. 1481) 	No safety concern
Structural class III						
Ethyl maltol isobutyrate	2252	852997-28-5 	Yes, SPET: 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 ^f (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; SPET: single-portion exposure technique

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

^b Step 2: One flavouring agent is in structural class II and one flavouring agent is in structural class III.

^c 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 SPET.

^d The margin of exposure was calculated based on the highest estimated daily dietary exposure calculated using the SPET.

^e The previously established ADI for maltol was withdrawn by the Committee.

^f 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 maltol 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.

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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 conditional⁴ 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].

⁴ "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 intakes⁵ 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

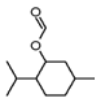
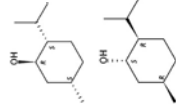
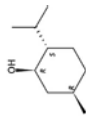
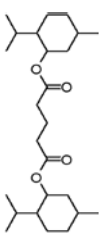
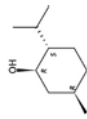
For eight previously evaluated flavouring agents in this group, additional studies of metabolism (No. 429); studies of acute toxicity (Nos 430, 1854 and 1856); and studies of genotoxicity (Nos 429, 430, 431, 445, 1856 and 1857) were available.

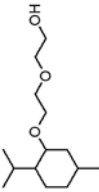
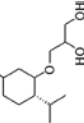
Conclusions

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

⁵ 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^{a,b}

Flavouring agent	No.	CAS no. and structure	Step 4 ^c	Step 5 ^d	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?		
Structural class I						
Menthyl formate	2246	2230-90-2 	No, SPET: 400	NR	NR	No safety concern
Menthyl propionate	2247	86014-82-6 	No, SPET: 600	NR	NR	No safety concern
<i>l</i> -Menthyl butyrate	2248	68366-64-3 	No, SPET: 300	NR	NR	No safety concern
<i>dl</i> -Isomenthol	2249	3623-52-7 	Yes, SPET: 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

Step 4: ^a		Step 5 ^d		Conclusion based on current estimated dietary exposure
Flavouring agent	No.	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?	
Menthol ^b	427	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.	No safety concern
Structural class III				
(±)-2-(2- <i>p</i> -Menthoxyl)ethanol	2251	Yes, SPET: 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.	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; SPET: single-portion exposure technique

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

^b 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.

^c 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 SPET or the MSDI method. The SPET gave the highest estimated dietary exposure in each case except for Nos 427 and 2250 where the MSDI value was higher.

^d The margin of exposure was calculated based on the highest estimated daily dietary exposure using either the SPET or the MSDI method.

^e 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*-methoxy)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.

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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

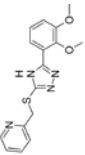
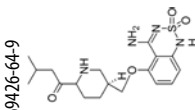
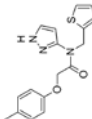
For previously evaluated flavouring agents in this group, studies of acute toxicity (No. 1566 and 1889), genotoxicity (No. 1566) and reproductive/developmental toxicity (No 2161) were available.

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^{a,b}

Flavouring agent	No.	CAS no. and structure	Step 4 ^c Does the highest dietary exposure estimate exceed the threshold of toxicological concern?	Step 5 ^d 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
Structural class III					
2-(((3-(2,3-Dimethoxyphenyl)-1 <i>H</i> -1,2,4-triazol-5-yl)thio)methyl)pyridine	2235	902136-79-2 	Yes, SPET: 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
(5 <i>S</i>)-1-(3-(((4-Amino-2,2-dioxido-1 <i>H</i> -benzol[1,2,6]thiadiazin-5-yl)oxy)methyl)piperidin-1-yl)-3-methylbutan-1-one	2236	1469426-64-9 	Yes, SPET: 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-Methoxyphenoxy)- <i>N</i> -(1 <i>H</i> -pyrazol-3-yl)- <i>N</i> -(thiophen-2-ylmethyl)acetamide	2237	1374760-95-8 	Yes, SPET: 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; SPET: single-portion exposure technique

^a Thirty-four flavouring agents in this group were previously evaluated by the Committee (Annex 1, references 178, 190, 211 and 220).
^b Step 2: All three flavouring agents are in structural class III.
^c 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.
^d 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

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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.
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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 CO₂ 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 acyclic branched-chain primary alcohols, aldehydes and acids used as flavouring agents^{a,b}

Flavouring agent	No.	CAS no. and structure	Step 4 ^c 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
Structural class I					
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

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

^b Step 2: Both flavouring agents are in structural class I.

^c 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

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

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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

The Committee wishes to thank Ms J. Odrowaz, Toronto, Canada, for her assistance in the preparation of the report.

FAO and WHO wish to acknowledge the significant contributions of the experts, as well as their institutions (where relevant), to the work of the eighty-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, diethylpyrocabamate, 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.
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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 ^a	The Committee was unable to complete the evaluation of AMC. 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	The Committee established an ADI “not specified” for basic methacrylate copolymer. 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 ^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. 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 ^b	The Committee considered the new data that had become available since the previous evaluation as well as previously evaluated studies and concluded that there are no reasons to revise the ADI and confirmed the previous ADI of 0–5 mg/kg bw.

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions
		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.
Lutein	R ^{c,d}	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. Based on the absence of toxicity in a wide range of studies, 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). <i>Meso</i>-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 <i>Tagetes erecta</i> and zeaxanthin (synthetic) was withdrawn.
Neutral methacrylate copolymer (NMC)	N, T	The Committee established an ADI “not specified” for NMC. The ADI “not specified” was made temporary because the specifications are tentative. 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. 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.
Sorbitol syrup	—	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.

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 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.
Spirulina extract	N, T	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 with spirulina extract and dried spirulina. The ADI “not specified” was made temporary due to the tentative nature of the specifications. 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.

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

^a The specifications were made tentative pending the completion of the safety evaluation of AMC.

^b 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.

^c The specifications for lutein esters from *Tagetes erecta* and zeaxanthin (synthetic) were maintained.

^d 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 ^a
Citric and fatty acid esters of glycerol	R, T ^b
Glycerol ester of wood rosin	R ^c
Modified starches	R ^d , T

R: existing specifications revised; T: tentative specifications

^a 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.

^b 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.

^c 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.

^d 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
Structural class I			
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
Structural class II			
(1-Methyl-2-(1,2,2- trimethylbicyclo[3.1.0]hex-3-ylmethyl)cyclopropyl)methanol	2254	N	No safety concern
Structural class III			
(±)-Bicyclo[2.2.1]hept-5-ene-2-carboxylic acid, ethyl ester	2255	N	No safety concern
Flavouring agent excluded at Step 1 of the Procedure			
<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
Structural class I			
Pinocaryyl isobutyrate	2242	N	No safety concern
Carvyl palmitate	2243	N	No safety concern
Structural class III			
6-Hydroxycarvone	2244	N	No safety concern
Flavouring agents not evaluated according to the revised Procedure			
(+)-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
Structural class III			
2-Pentylfuran	1491	M ^a	No safety concern
2-Heptylfuran	1492	M ^a	No safety concern
2-Decylfuran	1493	M ^a	No safety concern
3-Methyl-2-(3-methylbut-2-enyl)-furan	1494	M ^a	No safety concern
2,3-Dimethylbenzofuran	1495	M ^a	No safety concern
2,4-Difurfurylfuran	1496	M ^a	No safety concern
3-(2-Furyl)acrolein	1497	M ^a	No safety concern
2-Methyl-3(2-furyl)acrolein	1498	M ^a	No safety concern
3-(5-Methyl-2-furyl)prop-2-enal	1499	M ^a	No safety concern
3-(5-Methyl-2-furyl)butanal	1500	M ^a	No safety concern
2-Furfurylidene-butyraldehyde	1501	M ^a	No safety concern

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

M: existing specifications maintained

^a 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
Structural class I			
<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
Structural class II			
Maltol	1480	M	No safety concern ^a
Structural class III			
Ethyl maltol isobutyrate	2252	N	No safety concern

M: existing specifications maintained; N: new specifications

^a 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
Structural class I			
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>d,l</i> -Isomenthol	2249	N	No safety concern
Dimethyl glutarate	2250	N	No safety concern
Menthol	427	M	No safety concern
Structural class III			
(±)-2-[(2- <i>p</i> -Methoxy)ethoxy]ethanol	2251	N	No safety concern

M: existing specifications maintained; N: new specifications

^a 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
Structural class III			
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
Structural class I			
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 ^a
L-Malic acid	619	R ^b
Glutamyl-valyl-glycine	2123	R ^c

R: existing specifications revised

^a The CAS number was changed from 59259-38-0 to 61597-98-6 and the name to *L*-menthyl *L*-lactate.

^b The specification for specific rotation were removed.

^c 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
Furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers				
1519	2,5-Dimethyl-3-oxo-(2H)-fur-4-yl butyrate	93%	4-Hydroxy-2,5-dimethyl-3(2H)-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.
Linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters				
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.
Maltol and related substances				
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

86th 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

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)

Further information on these and other WHO publications can be obtained from
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Evaluation of certain food additives

This report represents the conclusions of a Joint FAO/WHO Expert Committee (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.

ISBN 978 92 4 121023 2



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Evaluation of certain food additives

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



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The World Health Organization was established in 1948 as a specialized agency of the United Nations serving as the directing and coordinating authority for international health matters and public health. One of WHO's constitutional functions is to provide objective and reliable information and advice in the field of human health, a responsibility that it fulfils in part through its extensive programme of publications. The Organization seeks through its publications to support national health strategies and address the most pressing public health concerns of populations around the world. To respond to the needs of Member States at all levels of development, WHO publishes practical manuals, handbooks and training material for specific categories of health workers; internationally applicable guidelines and standards; reviews and analyses of health policies, programmes and research; and state-of-the-art consensus reports that offer technical advice and recommendations for decision-makers. These books are closely tied to the Organization's priority activities, encompassing disease prevention and control, the development of equitable health systems based on primary health care, and health promotion for individuals and communities. Progress towards better health for all also demands the global dissemination and exchange of information that draws on the knowledge and experience of all WHO's Member countries and the collaboration of world leaders in public health and the biomedical sciences. To ensure the widest possible availability of authoritative information and guidance on health matters, WHO secures the broad international distribution of its publications and encourages their translation and adaptation. By helping to promote and protect health and prevent and control disease throughout the world, WHO's books contribute to achieving the Organization's principal objective – the attainment by all people of the highest possible level of health.

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Evaluation of certain food additives

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

*This report contains the collective views of an international groups of experts and
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WHO Library Cataloguing-in-Publication Data

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

(WHO technical report series ; no. 1000)

1.Food Additives - analysis. 2.Food Additives - toxicity. 3.Flavoring Agents. 4.Risk Assessment. I.World Health Organization. II.Food and Agriculture Organization of the United Nations. III.Joint FAO/WHO Expert Committee on Food Additives. Meeting (82nd: 2016, Geneva, Switzerland). IV.Series.

ISBN 978 92 4 121000 3

(NLM classification: WA 712)

ISBN 978 92 4 069586 3 (PDF)

ISSN 0512-3054

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Design: Rania Spatha (www.raniaspatha.com)

Printed in Malta

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¹ For use in infant formula and formula for special medical purposes intended for infants.

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Eighty-second meeting of the Joint FAO/WHO Expert Committee on Food Additives Geneva, 7–16 June 2016

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List of abbreviations

ADI	acceptable daily intake
AUC	area under the concentration–time curve
bw	body weight
CAS	Chemical Abstracts Service
CCCF	Codex Committee on Contaminants in Foods
CCFA	Codex Committee on Food Additives
CCFA48	Forty-eighth Session of the Codex Committee on Food Additives
CCFA49	Forty-ninth Session of the Codex Committee on Food Additives
CCFO	Codex Committee on Fats and Oils
CCNFSDU	Codex Committee on Nutrition and Foods for Special Dietary Uses
CITREM	citric and fatty acid esters of glycerol
C _{max}	maximum concentration
CYP	cytochrome P450
DNA	deoxyribonucleic acid
EFSA	European Food Safety Authority
EINECS	European Inventory of Existing Commercial Chemical Substances
EU	European Union
EU-16	Austria, Belgium, Czech Republic, Denmark, Finland, France, Greece, Ireland, Italy, Luxembourg, Netherlands, Portugal, Romania, Spain, Sweden, United Kingdom
F	filial generation (e.g. F ₁ , F ₂ , F ₃)
FAO	Food and Agriculture Organization of the United Nations
FSANZ	Food Standards Australia New Zealand
GC-FID	gas chromatography–flame ionization detection
GC-MS	gas chromatography–mass spectrometry
GRAS	Generally Recognized As Safe
GSFA	(Codex) General Standard for Food Additives
GST	glutathione S-transferase
HPLC	high-performance liquid chromatography
ICP-AES	inductively coupled plasma atomic emission spectrometry
ICP-MS	inductively coupled plasma mass spectrometry
ICP-OES	inductively coupled plasma optical emission spectrometry
Ig	immunoglobulin (e.g. IgA, IgG, IgM)
INS	International Numbering System for Food Additives
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LD ₅₀	median lethal dose
LOAEL	lowest-observed-adverse-effect level
ML	maximum level
MOE	margin of exposure

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

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

Safety evaluation of certain food additives. WHO Food Additives Series, No. 73, in preparation.

Specifications are issued separately by FAO under the title:

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

1. Introduction

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) met in Geneva from 7 to 16 June 2016. The meeting was opened by Dr Kazuaki Miyagishima, Director of the Department of Food Safety and Zoonoses of the World Health Organization (WHO), who welcomed participants on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations (FAO) and WHO. Dr Miyagishima noted that this year marks the sixtieth anniversary of JECFA, which is one of the longest standing expert committees at WHO and one of the best examples of collaboration between two agencies of the United Nations (UN). Providing scientific advice to Member States and to the Codex Alimentarius Commission is at the heart of the mandate of JECFA.

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

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

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

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

Dr Miyagishima noted that experts, whether from academia or from national agencies, are the critical asset of JECFA and ensure its neutrality and excellence. He reminded participants that they have been invited to this meeting as independent experts and not as representatives of their countries or

organizations. He also reminded them of the confidential nature of this meeting, which allows experts to freely express their opinions. He noted that the process by which FAO and WHO generate scientific advice is under global scrutiny, and, if an unfounded allegation is directed to its expert bodies and experts, WHO and FAO will do their best to defend their reputations. Dr Miyagishima closed by expressing his sincere gratitude to participants for providing their time and expertise to this important work and to their organizations for agreeing to put their experts at the disposal of JECFA.

1.1 Declarations of interests

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

1.2 Modification of the agenda

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

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

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

2. General considerations

As a result of the recommendations of the first Joint FAO/WHO Conference on Food Additives, held in September 1955 (1), there have been 81 previous meetings of the Committee (Annex 1). The present meeting was convened on the basis of a recommendation made at the seventy-ninth meeting (Annex 1, reference 220).

The tasks before the Committee were to:

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

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

The Codex Secretariat provided the Committee with an update on the work of CCFA since the eightieth meeting of JECFA (Annex 1, reference 223).

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

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

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

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

2.2 Principles governing the toxicological evaluation of compounds on the agenda

In making recommendations on the safety of food additives, the Committee took into consideration the principles established and contained in the publication, Environmental Health Criteria, No. 240, *Principles and methods for the risk assessment of chemicals in food*, published in 2009 (5).

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

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

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

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

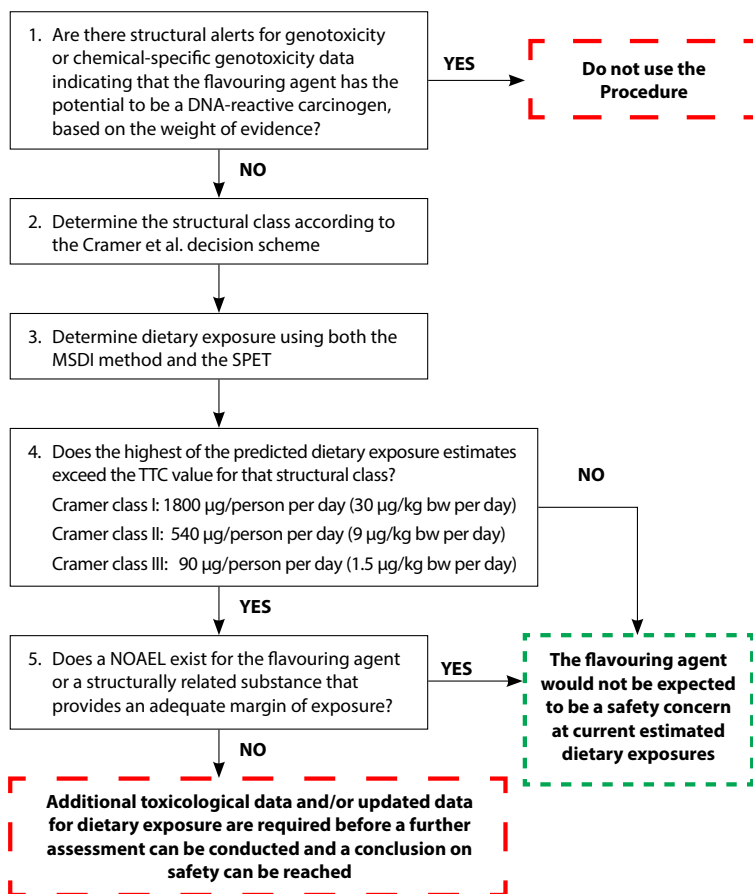
Another change is to add an initial question regarding genotoxicity and in consequence to delete step B5 (“Do the conditions of use result in an intake greater than 1.5 µg/day?”) from the Procedure. The Committee noted that this is the original United States Food and Drug Administration (USFDA) threshold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

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

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

Fig. 1

Revised Procedure for the Safety Evaluation of Flavouring Agents



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

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

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

2.2.2 Approach for prioritizing flavouring agents for re-evaluation

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

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

2.3 Food additive specifications and analytical methods

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

2.4 Flavour specifications

2.4.1 General inclusion of infrared spectra

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

2.4.2 Inclusion of chemical structures in the JECFA flavourings database

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

3. Specific food additives (other than flavouring agents)

The Committee evaluated one food additive for the first time and re-evaluated six others. In addition, the Committee evaluated the safety of three previously evaluated food additives for use in infant formula and formula for special medical purposes intended for infants. Twenty-two food additives (including 16 modified starches) were considered for revision of specifications only. Information on the safety evaluations and specifications is summarized in [Annex 2](#). Details of further toxicological studies and other information required for certain substances are summarized in [section 5](#).

3.1 Safety evaluations²

3.1.1 Allura Red AC

Explanation

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

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

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

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

Chemical and technical considerations

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

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

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

Biochemical aspects

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

Toxicological studies

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

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

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

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

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

In mice, no reproductive toxicity at dose levels up to 2520 mg/kg bw per day over two generations was reported [37]. In a two-generation reproductive toxicity study in rats [38], slight growth suppression was observed in F₁ and F₂ pups at 5.19% Allura Red AC in the diet, the highest concentration tested, as well as in the low-dose group of the F_{1B} generation. In the absence of detailed original data, the toxicological relevance of the observed “slight growth suppression” could not be assessed.

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

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

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

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

Observations in humans

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

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

Assessment of dietary exposure

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

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

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

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

Evaluation

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

A consolidated monograph was prepared.

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

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

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3.1.2 Carob bean gum

Explanation

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

At the request of CCFA at its Forty-seventh Session [1], the Committee evaluated the safety of carob bean gum for use as thickener in infant formula and formula for special medical purposes intended for infants in the context of the (therapeutic) dietary management of gastro-oesophageal reflux. The Committee notes that ADIs do not apply to infants up to the age of 12 weeks because they might be at risk at lower levels of exposure compared with older age groups. The proposed use level of carob bean gum is up to 10 000 mg/L for infant formula. The sponsor suggested that a typical use level would be 5000 mg/L.

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

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

Chemical and technical considerations

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

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

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

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

Biochemical aspects

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

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

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

Toxicological studies

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

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

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

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

Special studies

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

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

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

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

Observations in humans

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

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

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

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

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

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

Assessment of dietary exposure

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

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

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

Evaluation

The Committee previously assigned an ADI “not specified” to carob bean gum, but this does not apply to infants up to the age of 12 weeks because they might be at risk at lower levels of exposure compared with older age groups. Therefore, special considerations are required for this age group on a case-by-case basis, and toxicological testing strategies for additives to be used in infant formulas require different approaches, including studies involving exposure of very young animals. The Committee previously concluded that studies incorporating direct oral administration to neonatal animals are required for the evaluation of food additives in infant formula ([Annex 1](#), reference 220).

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

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

An addendum to the monograph was prepared.

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

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

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

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

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

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

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3.1.3 Lutein esters from *Tagetes erecta*

Explanation

Products extracted from *Tagetes erecta* containing lutein and its esters have been the subject of previous JECFA evaluations. At its thirty-first meeting (Annex 1, reference 77), the Committee prepared tentative specifications for xanthophylls obtained from *Tagetes erecta* petals, but no toxicological data were available, and no toxicological evaluation was performed. *Tagetes* extract containing lutein

esters at low concentrations was considered by the Committee at its fifty-fifth and fifty-seventh meetings ([Annex 1](#), references 149 and 154), and the tentative specifications were revised ([Annex 1](#), reference 151) and then superseded by full specifications ([Annex 1](#), reference 156). At its sixty-third meeting ([Annex 1](#), reference 173), the Committee evaluated biochemical data and the results of toxicological and human studies on *Tagetes* preparations with a high content of unesterified lutein (>80%) and established a group ADI of 0–2 mg/kg bw for lutein from *Tagetes erecta* and synthetic zeaxanthin.

At the seventy-ninth meeting ([Annex 1](#), reference 220), the Committee evaluated lutein esters from *Tagetes erecta*. The Committee noted that limited information was received on the manufacturing process for and composition of lutein esters from *Tagetes erecta* and therefore prepared tentative specifications for the product. Based on the available toxicological data, including newly submitted studies, and a dietary exposure assessment, the Committee concluded that there was no need to establish a numerical ADI. The Committee established a temporary ADI “not specified” for lutein esters from *Tagetes erecta*. The ADI was made temporary because the specifications for lutein esters from *Tagetes erecta* were tentative.

Chemical and technical considerations

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

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

Evaluation

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

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

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

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

The Committee at its seventy-ninth meeting considered establishing a group ADI “not specified” for lutein esters from *Tagetes erecta* that would include lutein from *Tagetes erecta* and synthetic zeaxanthin and related xanthophylls. The current Committee was not able to consider this aspect in detail and recommends that this be taken up at a future meeting.

An addendum to the monograph was not prepared.

3.1.4 Octenyl succinic acid (OSA)–modified gum arabic

Explanation

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

At its seventy-fourth meeting ([Annex 1](#), reference 205), the Committee evaluated new data on the hydrolysis of OSA-modified gum arabic and concluded that the results from the experiments did not unequivocally demonstrate that OSA-modified gum arabic hydrolyses completely in the stomach into gum arabic and OSA. Furthermore, the hydrolysis experiments showed inconsistencies with the reported stability of OSA-modified gum arabic in food. Therefore, the Committee requested that additional data be provided (data on the stability of OSA-modified gum arabic in food and data showing complete hydrolysis in the gastrointestinal tract) by the end of 2013 for further evaluation. The temporary

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

At the seventy-seventh meeting ([Annex 1](#), reference 214), the Committee evaluated a new study on the hydrolysis of OSA-modified gum arabic in simulated gastric fluid, simulated intestinal fluid and water. The Committee noted that complete hydrolysis of OSA-modified gum arabic under neutral pH conditions in simulated intestinal fluid or water, as reported in the study, was not in accordance with the claimed stability of the OSA ester linkage in aqueous solutions at the pH range of foods and beverages [1]. Considering that spontaneous hydrolysis of OSA-modified gum arabic in water was unlikely to occur, the Committee doubted the validity of the observed hydrolysis in the presence of gastrointestinal enzymes. As the study did not unequivocally demonstrate that OSA-modified gum arabic hydrolyses completely in the stomach into gum arabic and OSA, the validity of using toxicological data on gum arabic in the evaluation of OSA-modified gum arabic was not confirmed. The Committee also reviewed data on the stability of OSA-modified gum arabic in food. Although these data demonstrated that OSA-modified gum arabic provided a stable emulsion in the two model food systems evaluated, the data did not unequivocally demonstrate that the OSA-modified gum arabic, at the molecular level, is stable in food and beverages. The Committee decided to retain the temporary ADI “not specified”, pending submission of additional data on the stability of OSA-modified gum arabic in food. The specifications were made tentative, pending submission of information on an analytical method to measure the degree of substitution and the results of the analysis of at least five commercially available batches.

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

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

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

Chemical and technical considerations

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

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

Evaluation of the newly submitted studies

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

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

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

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

Assessment of dietary exposure

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

Evaluation

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

No addendum to the monograph was prepared.

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

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3.1.5 Pectin

Explanation

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

At its seventy-ninth meeting, the Committee evaluated data relevant to the safety of pectin in infant formula, noting that the group ADI does not apply to infants up to the age of 12 weeks because they might be at risk at lower levels of exposure compared with older age groups. The Committee concluded that estimated exposure to pectin from its use in infant formula (1100 mg/kg bw per day) at the then proposed level of 0.5% (5000 mg/L) was in the region of the NOAEL of pectin (847 mg/kg bw per day) and close to the lowest-observed-adverse-effect level (LOAEL) (3013 mg/kg bw per day), based on decreased feed intake and body weight gain in a neonatal pig study. Using the NOAEL from this study, the MOEs were estimated to be 0.9 for infants with median energy intake and 0.8 for infants with high (95th percentile) energy intake. The Committee therefore concluded that the use of pectin in infant formulas at the maximum proposed use level (0.5%) was of concern and requested additional data to support the safety evaluation of pectin in infant formula, including an explanation for the decreased feed intake and body weight gain in neonatal pigs. In addition, the Committee requested data on levels of lead when the additive is intended for use in infant formula.

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

Chemical and technical considerations

Pectin is a complex heteropolysaccharide that consists mainly of the partial methyl esters of polygalacturonic acid and their sodium, potassium, calcium and ammonium salts. It is obtained by aqueous extraction of appropriate edible plant

material, usually citrus fruits or apples. The average molecular weight of pectin used in food will vary depending upon the pectin source and processing and is expected to range from 100 to 200 kDa.

Pectin is used in infant formula as a thickener to increase the viscosity of the formula and as a stabilizer to maintain the homogeneity of the formula throughout its shelf life.

Biochemical aspects

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

Pectin-derived acidic oligosaccharides (pAOS) are a product of the digestion of food-grade pectin and consist of small polymers predominantly of molecular weight of no more than 3800 Da. Manufactured pAOS is similar to products formed from pectin in the gastrointestinal tract. The Committee at the seventy-ninth meeting concluded that studies on pAOS can support conclusions reached on the basis of data from studies that have used pectin.

Toxicological studies

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

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

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

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

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

Observations in humans

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

Assessment of dietary exposure

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

Infant formula consumption estimates were derived from mean estimated energy requirements for fully formula-fed infants. It should be noted that the energy requirements of formula-fed infants are greater than those of

breastfed infants, although this disparity decreases with increasing age. A further exposure scenario was considered, using high (95th percentile) daily energy intakes reported for formula-fed infants. The highest reported 95th percentile energy intakes per kilogram body weight were for infants aged 14–27 days. For all dietary exposure estimates, a common energy density of formula of 67 kcal/100 mL (280 kJ/100 mL) was used to convert energy needs to the volume of formula ingested daily.

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

Evaluation

The Committee previously assigned a group ADI “not specified” to pectin and amidated pectins, but this group ADI does not apply to infants up to the age of 12 weeks because they might be at risk at lower levels of exposure compared with older age groups. Therefore, special considerations are required for this age group on a case-by-case basis, and toxicological testing strategies for additives to be used in infant formulas require different approaches, including studies involving exposure of very young animals.

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

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

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

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

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

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

An addendum to the monograph was prepared.

The Committee at its seventy-first meeting ([Annex 1](#), reference 196) had prepared specifications for pectins. The Committee discussed limits on lead specifications for this and the other food additives for use in infant formula that were on the agenda, as described in section 2.3.3. At the present meeting, the specifications for pectin were revised to lower the limit for lead from 5 to 2 mg/kg for general use, to introduce a limit for lead of 0.5 mg/kg for use in infant formula and to update the method descriptions for the determination of lead and sample preparation for residual solvents.

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3.1.6 Quinoline Yellow

Explanation

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

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

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

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

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

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

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

Chemical and technical considerations

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

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

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

Quinoline Yellow

Biochemical aspects

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

Toxicological studies

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

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

Observations in humans

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

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

D&C Yellow No. 10

Biochemical aspects

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

Toxicological studies

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

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

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

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

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

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

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

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

Assessment of dietary exposure

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

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

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

Evaluation

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

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

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

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

A monograph was prepared.

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

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

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

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3.1.7 Rosemary extract

Explanation

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

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

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

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

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

Chemical and technical considerations

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

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

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

Biochemical aspects

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

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

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

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

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

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

Toxicological studies

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

Rosemary extracts and an isolated extract constituent, carnosic acid, have low acute oral toxicity in rats and mice. The oral median lethal dose (LD₅₀) was greater than 2000 mg/kg bw for rosemary extracts administered by gavage to rats [12, 13] and was 7100 mg/kg bw for carnosic acid administered by gavage to mice [14].

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

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

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

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

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

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

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

Observations in humans

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

Assessment of dietary exposure

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

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

Evaluation

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

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

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

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

A monograph was prepared.

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

A Chemical and Technical Assessment was prepared for rosemary extract.

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3.1.8 Steviol glycosides

Explanation

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

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

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

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

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

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

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

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

Chemical and technical considerations

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

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

Rebaudioside A from Multiple Gene Donors Expressed in *Yarrowia lipolytica* is produced by fermentation of a genetically modified strain of *Yarrowia lipolytica* to express the *Stevia rebaudiana* Bertoni metabolic pathway. It is composed of at least 95% (on the anhydrous basis) of rebaudioside A (13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)-oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester; CAS No. 58543-16-1; chemical formula C₄₄H₇₀O₂₃), with minor amounts of other steviol glycosides.

Biochemical aspects

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

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

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

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

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

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

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

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

Toxicological studies

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

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

Observations in humans

No new human studies were available.

Assessment of dietary exposure

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

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

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

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

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

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

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

Evaluation

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

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

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

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

An addendum to the monograph was prepared.

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

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

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

A Chemical and Technical Assessment was prepared.

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

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3.1.9 Tartrazine

Explanation

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

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

Chemical and technical considerations

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

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

Biochemical aspects

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

Toxicological studies

Tartrazine is of low acute toxicity, with an LD₅₀ value of greater than 2000 mg/kg bw [6].

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Observations in humans

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

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

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

Assessment of dietary exposure

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

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

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

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

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

Evaluation

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

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

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

An addendum to the monograph was prepared.

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

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

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3.1.10 Xanthan gum

Explanation

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

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

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

Chemical and technical considerations

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

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

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

Biochemical aspects

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

Toxicological studies

At the thirtieth meeting ([Annex 1](#), references 73 and 74), the Committee noted that in mice, rats and dogs, xanthan gum exhibited low oral toxicity, with LD₅₀ values ranging from greater than 1000 to greater than 45 000 mg/kg bw [1, 9, 10]. In short-term toxicity studies in animals, effects occurred mainly in the intestine at doses above 700 mg/kg bw per day. These included faecal bulking and water binding, with some increases in intestinal tissue mass. At higher doses, reduced nutrient absorption was reported, which explained reduced weight gain and lower liver weights. Other organ weights were unchanged, and no gross morphological or histological abnormalities were reported. In dogs fed a diet containing xanthan gum for 12 weeks, stool softening was observed at doses above 250 mg/kg bw per day, and occasional diarrhoea was seen at 1000 mg/kg bw per day [11].

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

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

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

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

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

Special studies in neonatal pigs

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

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

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

Observations in humans

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

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

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

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

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

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

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

Post-marketing surveillance data were collected by one manufacturer over a 5-year period (June 2010 through May 2015) during which distribution of the hydrolysed powder containing xanthan gum (reconstituted at xanthan gum concentrations up to 1000 mg/L) and the ready-to-feed equivalent (without xanthan gum) equalled 105 million litres (providing exposure of a total of 131 million patient treatment days³ from the manufacturer's products). The data show that consumption of formula containing xanthan gum is not associated with an increased rate of adverse events. The rate for any adverse event was less than one report per 10 000 patient treatment days. Overall, the post-market surveillance data provide additional support for the safe use of xanthan gum in infant formula, including specialized formula consumed by infants with protein allergy [33].

Assessment of dietary exposure

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

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

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

Evaluation

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

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

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

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

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

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

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

A consolidated monograph was prepared.

At the present meeting, the Committee reviewed the specifications for xanthan gum. The Committee discussed limits on lead specifications for this and the other food additives for use in infant formula that were on the agenda, as described in section 2.3.3. The Committee maintained the limit for lead in xanthan gum at 2 mg/kg for general use and introduced a limit for lead of 0.5 mg/kg for use in infant formula.

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

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

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3.2 Revision of specifications

3.2.1 Aspartame

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

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

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

3.2.2 Cassia gum

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

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

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

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

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

3.2.3 Citric and fatty acid esters of glycerol (CITREM)

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

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

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

3.2.4 Modified starches

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

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

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

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

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

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

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

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

3.2.5 Octanoic acid

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

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

3.2.6 Starch sodium octenyl succinate

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

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

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

3.2.7 Total colouring matters

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

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

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

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

4. Flavouring agents

4.1 Flavouring agents evaluated by the Procedure for the Safety Evaluation of Flavouring Agents^{4,5}

Assignment to structural class

Five groups of flavouring agents were evaluated using the Procedure for the Safety Evaluation of Flavouring Agents, as outlined in [Fig. 2 \(Annex 1, references 116, 122, 131, 137, 143, 149, 154, 160, 166, 173 and 178\)](#). In applying the Procedure, the chemical is first assigned to a structural class as identified by the Committee at its forty-sixth meeting ([Annex 1, reference 122](#)). The structural classes are as follows:

- *Class I.* Flavouring agents that have simple chemical structures and efficient modes of metabolism that would suggest a low order of toxicity by the oral route.
- *Class II.* Flavouring agents that have structural features that are less innocuous than those of substances in class I but are not suggestive of toxicity. Substances in this class may contain reactive functional groups.
- *Class III.* Flavouring agents that have structural features that permit no strong initial presumption of safety or may even suggest significant toxicity.

A key element of the Procedure involves determining whether a flavouring agent and the product(s) of its metabolism are innocuous and/or endogenous substances. For the purpose of the evaluations, the Committee used the following definitions, adapted from the report of its forty-sixth meeting ([Annex 1, reference 122](#)):

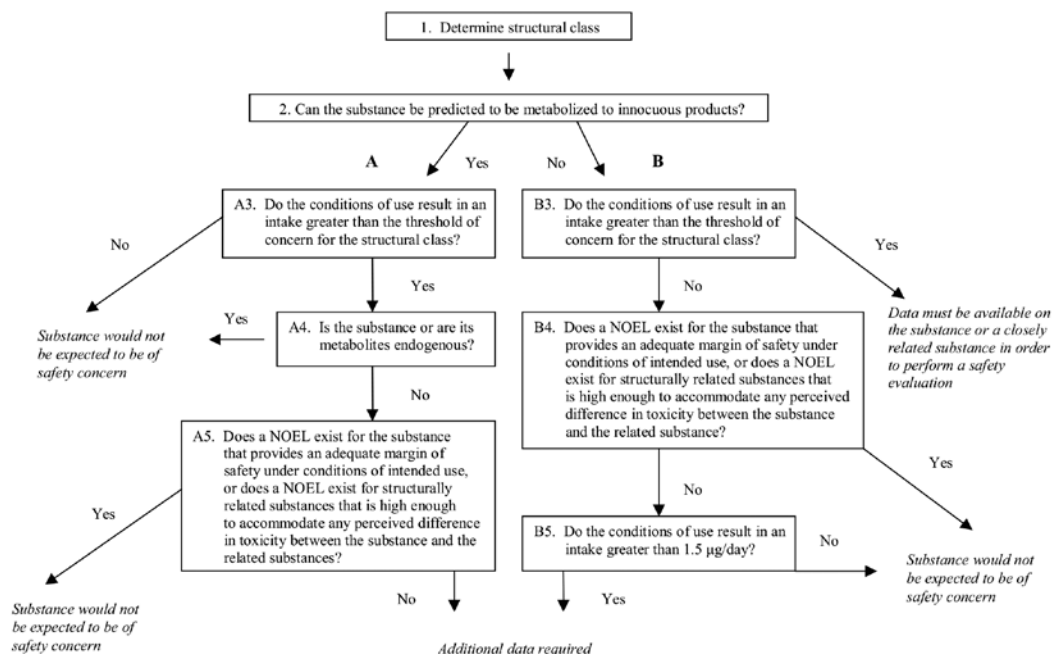
- *Innocuous metabolic products* are defined as products that are known or readily predicted to be harmless to humans at the estimated dietary exposure to the flavouring agent.
- *Endogenous substances* are intermediary metabolites normally present in human tissues and fluids, whether free or conjugated; hormones and other substances with biochemical or physiological regulatory functions are not included. The estimated dietary exposure

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

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

Fig. 2

Procedure for the Safety Evaluation of Flavouring Agents



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

Assessment of dietary exposure

Maximized survey-derived intake (MSDI)

Estimates of the dietary exposure to flavouring agents by populations are based on annual volumes of production. These data were derived from surveys in Europe, Japan and the USA. Manufacturers were requested to exclude use of flavouring agents in pharmaceutical, tobacco or cosmetic products when compiling these data. When using these production volumes to estimate dietary exposures, a correction factor of 0.8 is applied to account for under-reporting.

$$\text{MSDI } (\mu\text{g/day}) = \frac{\text{annual volume of production (kg)} \times 10^9 \text{ } (\mu\text{g/kg})}{\text{population of consumers} \times 0.8 \times 365 \text{ days}}$$

The population of consumers was assumed to be 41×10^6 in Europe, 13×10^6 in Japan and 31×10^6 in the USA.⁶

Single-portion exposure technique (SPET)

The SPET was developed by the Committee at its sixty-seventh meeting ([Annex 1](#), reference 184) to account for presumed patterns of consumer behaviour with respect to food consumption and the possible uneven distribution of dietary exposures among consumers of foods containing flavouring agents. It is based on reported use levels supplied by the industry. This single portion-derived estimate was designed to account for individuals' brand loyalty to food products and for niche products that would be expected to be consumed by only a small proportion of the population. Its use in the Procedure was endorsed at the sixty-ninth meeting of the Committee ([Annex 1](#), reference 190) to render the safety assessment more robust, replacing the sole use of MSDI estimates with the higher of the highest MSDI or the SPET estimate as the exposure estimate in the decision-tree. The Committee also agreed that it would not be necessary to re-evaluate flavouring agents that had already been assessed previously using the Procedure.

The SPET provides an estimate of dietary exposure for an individual who consumes a specific food product containing the flavouring agent every day. The SPET combines an average (or usual) added use level provided by the flavour industry with a standard portion size from 75 predefined food categories as described by the Committee at its sixty-seventh meeting. The standard portion is taken to represent the mean food consumption for consumers of these food categories. Among all the food categories with a reported use level, the calculated dietary exposure from the single food category leading to the highest dietary exposure from one portion is taken as the SPET estimate:

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

The highest result is used in the evaluation.

The use level data provided by industry for each flavouring agent evaluated at this meeting and used in the SPET calculations are available on the WHO JECFA website at <http://www.who.int/foodsafety/publications/jecfa/en/>.

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

Consideration of combined intakes from use as flavouring agents

The safety assessment of possible combined intakes of flavouring agents was based on the presence of common metabolites or a homologous series (as proposed at the sixty-eighth meeting; [Annex 1](#), reference 187) and using the MSDI exposure assessment (as proposed at the sixty-ninth meeting; [Annex 1](#), reference 190).

4.1.1 Alicyclic, alicyclic-fused and aromatic-fused ring lactones***Introduction***

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

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

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

Assessment of dietary exposure

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

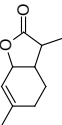
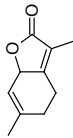
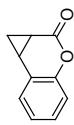
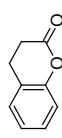
Dietary exposures were estimated using the MSDI method and the SPET. The highest estimated dietary exposure for each flavouring agent is reported in [Table 1](#). The estimated daily dietary exposure is highest for 2-(2-hydroxy-4-methyl-3-cyclohexenyl)propionic acid gamma-lactone (No. 2223) (300 µg/day,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

Absorption, distribution, metabolism and elimination

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

Table 1

Summary of the results of the safety evaluations of alicyclic, alicyclic-fused and aromatic-fused ring lactones used as flavouring agents^{a,b,c}

Flavouring agent	No.	CAS No. and structure	Step A3 ^d	Step A4	Step A5 ^e	Comments on predicted metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
			Does estimated dietary exposure exceed the threshold of concern?	Is the flavouring agent or are its metabolites endogenous?				
Structural class III								
2-(2-Hydroxy-4-methyl-3-cyclohexenyl)propionic acid gamma-lactone	2223	57743-63-2 	Yes, SPET: 300	No	Yes. The NOAEL of 1 mg/kg bw per day for the structurally related dehydromenthofulactone in a 90-day study in rats [5] is 200 times the estimated dietary exposure to No. 2223 when used as a flavouring agent.	Note 1	Dehydromenthofulactone (No. 1163) 	No safety concern
2-(2-Hydroxyphenyl)-cyclopropanecarboxylic acid delta-lactone	2224	5617-64-1 	Yes, SPET: 250	No	Yes. The NOAEL of 150 mg/kg bw per day for the structurally related dihydrocoumarin in a 90-day study in rats [6] is 38 000 times the estimated dietary exposure to No. 2224 when used as a flavouring agent.	Note 2	Dihydrocoumarin (No. 1171) 	No safety concern

^a Sixteen flavouring agents in this group were previously evaluated by the Committee (Annex 1, reference 160).

^b Step 1: Both flavouring agents are in structural class III.

^c Step 2: Both flavouring agents in this group can be predicted to be metabolized to innocuous products.

^d The threshold for human dietary exposure for structural class III is 90 µg/day. All dietary exposure values are expressed in µg/day. The dietary exposure values listed represent the highest values calculated by either the SPET or MSDI method. The SPET gave the highest estimated dietary exposure in each case.

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

Notes:

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

fused ring lactones has previously been described in the report of the sixty-first meeting ([Annex 1](#), reference 166). No additional information was available for this meeting.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents, the Committee assigned both flavouring agents (Nos 2223 and 2224) to structural class III [4].

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

Step A3. The highest estimated dietary exposures for the two flavouring agents are above the threshold of concern (i.e. 90 µg/day for class III). Accordingly, the evaluation of these flavouring agents proceeded to step A4.

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

Step A5. For 2-(2-hydroxy-4-methyl-3-cyclohexenyl)propionic acid gamma-lactone (No. 2223), the NOAEL⁷ of 1 mg/kg bw per day for the structurally related dehydromenthofurolactone (No. 1163) obtained from a 90-day study in rats [5] ([Annex 1](#), reference 167) provides an adequate MOE of 200 in relation to the highest estimated dietary exposure to No. 2223 (SPET = 300 µg/day or 5 µg/kg bw per day) when used as a flavouring agent. The Committee therefore concluded that 2-(2-hydroxy-4-methyl-3-cyclohexenyl)propionic acid gamma-lactone (No. 2223) would not pose a safety concern at current estimated dietary exposures.

For 2-(2-hydroxyphenyl)cyclopropanecarboxylic acid delta-lactone (No. 2224), the NOAEL⁷ of 150 mg/kg bw per day for the structurally related dihydrocoumarin (No. 1171) obtained from a 90-day study in rats [6] ([Annex 1](#), reference 167) provides an adequate MOE of 38 000 in relation to the estimated dietary exposure to No. 2224 (SPET = 250 µg/day or 4 µg/kg bw per day) when used as a flavouring agent. The Committee therefore concluded that 2-(2-hydroxyphenyl)cyclopropanecarboxylic acid delta-lactone (No. 2224) would not pose a safety concern at current estimated dietary exposures.

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

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

Consideration of combined intakes from use as flavouring agents

The two additional flavouring agents in this group of alicyclic, alicyclic-fused and aromatic-fused ring lactones have low MSDI values (0.01–0.09 µg/day). The Committee concluded that consideration of combined intakes is not necessary, because the additional flavouring agents would not contribute significantly to the combined intake of this group.

Consideration of secondary components

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

Conclusions

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

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

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

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

An addendum to the monograph was prepared.

References

1. Nijssen LM, Ingen-Visscher CAV, Donders JJH. Volatile compounds in food 16.1 [database]. Zeist: TNO Triskelion; 2015 (<http://www.vcf-online.nl/VcfHome.cfm>).
2. 2010 IOFI global poundage survey. Geneva: International Organization of the Flavor Industry; 2013. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
3. International Organization of the Flavor Industry. Interim inquiry on volume use and added use levels for flavoring agents to be presented at the JECFA 82nd meeting. Private communication to the

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

4. Cramer GM, Ford RA, Hall RL. Estimation of toxic hazard – a decision tree approach. *Food Cosmet Toxicol.* 1978;16:255–76.
5. Voss KA. 90-day dietary toxicity study of SRA 84-11 in Sprague-Dawley rats (dehydromenthofurolactone). Unpublished report to the Flavor and Extract Manufacturers Association; 1985. Submitted to WHO by the International Organization for the Flavor Industry, Brussels, Belgium.
6. Toxicology and carcinogenesis studies of 3,4-dihydrocoumarin (CAS 119-84-6) in F344/N rats and B6C3F1 mice (gavage studies). Research Triangle Park (NC): United States Department of Health and Human Services, Public Health Service, National Institutes of Health, National Toxicology Program; 1993 (NTP Technical Report Series No. 423).

4.1.2 Aliphatic and aromatic amines and amides

Introduction

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

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

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

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

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

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

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

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

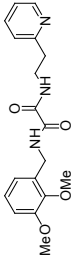
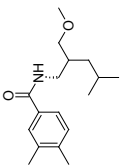
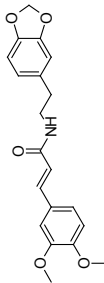
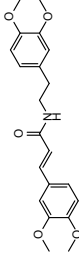
Assessment of dietary exposure

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

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

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

Table 2
Summary of the results of the safety evaluations of aliphatic and aromatic amines and amides used as flavouring agents^{a,b,c}

Flavouring agent	No.	CAS No. and structure	Step B3 ^d Does estimated dietary exposure exceed the threshold of concern?	Follow-on from step B3 ^e Are additional data available for the flavouring agent with an estimated dietary exposure exceeding the threshold of concern?	Comments on predicted metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
Structural class III							
<i>N</i> -(2,3-Dimethoxybenzyl)- <i>N</i> 2-(2-(pyridin-2-yl)ethyl)-oxalamide	2225	851670-40-18 	Yes, SPET: 2 000	Yes. No. 2225 is non-genotoxic in bacteria, and the NOAEL of 140 mg/kg bw per day (the highest dose tested) in a 28-day study in rats [12] is 4 200 times the estimated dietary exposure to No. 2225 when used as a flavouring agent.	Note 1	–	No safety concern
(<i>R</i>)- <i>N</i> -(1-Methoxy-4-methylpentan-2-yl)-3,4-dimethylbenzamide	2226	851669-60-8 	Yes, SPET: 800	Yes. No. 2226 is non-genotoxic in bacteria and in mammalian cells in vitro and in vivo, and the NOAEL of 100 mg/kg bw per day (the highest dose tested) in a 28-day study in rats [16] is 7 700 times the estimated dietary exposure to No. 2226 when used as a flavouring agent.	Note 2	–	No safety concern
(<i>E</i>)- <i>N</i> -(2-(1,3-Benzodioxol-5-yl)ethyl)-3-(3,4-dimethoxyphenyl)prop-2-enamide	2227	125187-30-6 	Yes, SPET: 400	Yes. No. 2227 and the structurally related <i>N</i> -(2-(3,4-dimethoxyphenyl)-ethyl)-3,4-dimethoxycinnamic acid amide (No. 1777) are non-genotoxic in bacteria, and the NOAEL of 69 mg/kg bw per day for No. 1777 in a 90-day study in rats [18] is 9 900 times the estimated dietary exposure to No. 2227 when used as a flavouring agent.	Note 1	<i>N</i> -(2-(3,4-Dimethoxyphenyl)ethyl)-3,4-dimethoxycinnamic acid amide (No. 1777) 	No safety concern

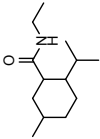
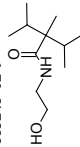
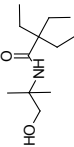
Step B3 ^d		Follow-on from step B3 ^e		Comments on predicted metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
Flavouring agent	No.	Does estimated dietary exposure exceed the threshold of concern?	Are additional data available for the flavouring agent with an estimated dietary exposure exceeding the threshold of concern?			
(E)-3-Benzyl-3,4-dioxol-5-yl-N,N-diphenyl-2-propenamide	2228	Yes, SPET: 100	Yes, No. 2228 is non-genotoxic in bacteria, and the NOAEL of 490 mg/kg bw per day (the highest dose tested) in a 90-day study in rats [20] is 245 000 times the estimated dietary exposure to No. 2228 when used as a flavouring agent.	Note 1	–	No safety concern
N-Ethyl-5-methyl-2-(methyl-2-ethenyl)-cyclohexanecarboxamide	2229	Yes, SPET: 15 000	Yes, for the structurally related N-ethyl-2-isopropyl-5-methylcyclohexanecarboxamide (No. 1601). This compound is of low acute toxicity, and from four short-term toxicity studies available, the overall NOAEL of 8 mg/kg bw per day in a 28-day study in rats [21] is 32 times the SPET estimate and 40 million times the MSD when No. 2229 is used as a flavouring agent.	Note 1	N-Ethyl 2-isopropyl-5-methyl-cyclohexanecarboxamide (No. 1601)	Additional data required to complete evaluation
						
N-Ethyl-2,2-diisopropylbutanamide	2005	Yes, SPET: 27 000	No	Note 1	–	Additional data required to complete evaluation
N-(2-Hydroxyethyl)-2,3-dimethyl-2-isopropylbutanamide	2010	Yes, SPET: 48 000	No	Notes 1 and 3	–	Additional data required to complete evaluation
						

Table 2 (continued)

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

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

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

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

^d The threshold for human dietary exposure for structural class III is 90 µg/day. All dietary exposure values are expressed in µg/day. The dietary exposure values listed represent the highest daily dietary exposures calculated by either the SPEI or the MSDI method. The SPEI gave the highest estimated dietary exposure in each case.

^e The MOEs were calculated based on the estimated dietary exposure calculated by the SPEI. In cases where the resulting MOE was relatively low, a comparison with the MSDI was also made.

Notes:

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

Absorption, distribution, metabolism and elimination

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

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

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

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

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

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

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

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

Application of the Procedure for the Safety Evaluation of Flavouring Agents

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

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

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

Step B3. The highest estimated dietary exposures for all eight flavouring agents in this group are above the threshold of concern (i.e. 90 µg/day for class III). Accordingly, data must be available on these flavouring agents or closely related substances to perform a safety evaluation.

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

In accordance with the Procedure, additional data were evaluated for Nos 2225–2229, 2005, 2010 and 2011, as their estimated dietary exposures exceeded the threshold of concern for structural class III (90 µg/day).

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

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

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

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

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

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

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

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

Consideration of combined intakes from use as flavouring agents

The five additional flavouring agents in the group of aliphatic and aromatic amines and amides have low MSDIs (0.01–0.03 µg/day). The Committee concluded that consideration of combined intakes is not necessary, because the additional flavouring agents would not contribute significantly to the combined intake of this flavouring group.

Consideration of additional data on previously evaluated flavouring agents

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

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

Conclusion

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

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

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

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

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

An addendum to the monograph was prepared.

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4.1.3 Aliphatic secondary alcohols, ketones and related esters

Introduction

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

2221). These flavouring agents included two unsaturated secondary alcohols (Nos 2218 and 2220), one saturated secondary alcohol (No. 2221) and three unsaturated ketones (Nos 2216, 2217 and 2219). The evaluations were conducted using the Procedure for the Safety Evaluation of Flavouring Agents ([Annex 1](#), reference 131). None of these flavouring agents has previously been evaluated by the Committee.

The Committee previously evaluated 63 other members of this group of flavouring agents at its fifty-ninth, sixty-ninth and seventy-third meetings ([Annex 1](#), references 160, 190 and 202). The Committee concluded that all 63 flavouring agents were of no safety concern at estimated dietary exposures.

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

Assessment of dietary exposure

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

Dietary exposures were estimated using the MSDI method and the SPET. The highest estimated dietary exposure for each flavouring agent is reported in [Table 3](#). The estimated daily dietary exposure is highest for (±)-1-cyclohexylethanol (No. 2221) (3000 µg/day, the SPET value obtained from hard candy) [4]. For the other flavouring agents, dietary exposures as SPET or MSDI estimates range from 0.01 to 2000 µg/day, with the SPET yielding the highest estimate in each case.

Absorption, distribution, metabolism and elimination

Information on the absorption, distribution, metabolism and elimination of flavouring agents belonging to the group of aliphatic secondary alcohols, ketones and related esters has previously been described in the monographs of the fifty-ninth and sixty-ninth meetings ([Annex 1](#), references 161 and 191). No additional information was available for this meeting.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the above-mentioned flavouring agents, the Committee assigned all six flavouring agents (Nos 2216–2221) to structural class II [5].

Table 3
Summary of the results of the safety evaluations of aliphatic secondary alcohols, ketones and related esters used as flavouring agents^{a,b,c}

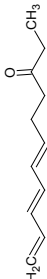
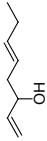
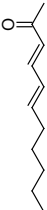
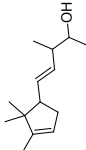
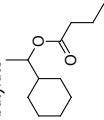
Step A3 ^d		Step A4		Step A5 ^e		Conclusion based on current estimated dietary exposure		
Does estimated dietary exposure exceed the threshold of concern?	Is the flavouring agent or are its metabolites endogenous?	Adequate margin of exposure for the flavouring agent or related substances?	Comments on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

Table 3 (continued)

Step A3 ^a		Step A4	Step A5 ^c		Comments on predicted metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
Flavouring agent	No.	Does estimated dietary exposure exceed the threshold of concern?	Is the flavouring agent or are its metabolites endogenous?	Adequate margin of exposure for the flavouring agent or related substances?			
(±)-1-Cyclohexylethanol	2221	Yes, SPET: 3 000	No	Yes. The NOAEL of 300 mg/kg bw per day for the structurally related 1-cyclohexylethyl butyrate in a 28-day study in rats [7] is 6 000 times the estimated dietary exposure to No. 2221 when used as a flavouring agent.	Notes 2 and 3	1-Cyclohexylethyl butyrate 	No safety concern

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

^a Sixty-three flavouring agents in this group were previously evaluated by the Committee (Annex 1, references 160, 190 and 202).
^b Step 1: All six flavouring agents are in structural class II.
^c Step 2: All six flavouring agents in this group can be predicted to be metabolized to innocuous products.
^d The threshold for human dietary exposure for structural class II is 540 µg/day. All dietary exposure values are expressed in µg/day. The dietary exposure values listed represent the highest values calculated by either the SPET or MSDI method. The SPET gave the highest estimated dietary exposure in each case.
^e The MOEs were calculated based on estimated dietary exposure calculated using the SPET.

Notes:

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

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

Step A3. The highest estimated dietary exposures for four of the flavouring agents (Nos 2217–2220) are below the threshold of concern (i.e. 540 µg/day for class II). The Committee therefore concluded that none of these four flavouring agents would pose a safety concern at current estimated dietary exposures. Two of the flavouring agents (Nos 2216 and 2221) have estimated dietary exposures greater than the threshold of concern (i.e. 540 µg/day for class II). Accordingly, the evaluation of these flavouring agents proceeded to step A4.

Step A4. These flavouring agents (Nos 2216 and 2221) and their metabolites are not endogenous, and therefore their evaluations proceeded to step A5.

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

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

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

Consideration of combined intakes from use as flavouring agents

The six additional flavouring agents in this group of aliphatic secondary alcohols, ketones and related esters have low MSDI values (0.01–32 µg/day). The Committee concluded that consideration of combined intakes is not necessary, because the additional flavouring agents would not contribute significantly to the combined intake of this group.

Consideration of secondary components

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

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

Conclusions

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

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

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

An addendum to the monograph was prepared.

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4.1.4 Cinnamyl alcohol and related substances

Introduction

The Committee evaluated an additional five flavouring agents belonging to the group of cinnamyl alcohol and related substances (Nos 2211–2215). These flavouring agents included two esters (one with an additional aldehyde functional group: No. 2211; and one with an additional alcohol functional group: No. 2213), an aldehyde with a methylenedioxyphenyl functional group (No. 2212) and two acetals (Nos 2214 and 2215). The evaluations were conducted using the Procedure for the Safety Evaluation of Flavouring Agents ([Annex 1](#), reference 131). None of these flavouring agents has previously been evaluated by the Committee.

The Committee previously evaluated 55 other members of this group of flavouring agents at its fifty-fifth meeting ([Annex 1](#), reference 149). The Committee concluded that all 55 flavouring agents in that group were of no safety concern at estimated dietary exposures.

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

Assessment of dietary exposure

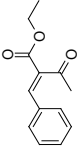
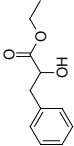
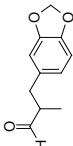
The total annual volumes of production of the five flavouring agents belonging to the group of cinnamyl alcohol and related substances are approximately 32 kg in Europe, 0.1 kg in the USA and 31 kg in Japan [2].

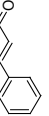
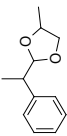
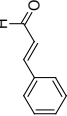
Dietary exposures were estimated using both the MSDI method and the SPET. The highest estimated dietary exposure for each flavouring agent is reported in [Table 4](#). The estimated daily dietary exposure is highest for 3-(3,4-methylenedioxyphenyl)-2-methylpropanal (No. 2212) (3000 µg/day, the SPET value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

Absorption, distribution, metabolism and elimination

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

Table 4
Summary of the results of the safety evaluations of cinnamyl alcohol and related substances used as flavouring agents^{a,b,c}

		Step A3/B3 ^d Does estimated dietary exposure exceed the threshold of concern?	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 ^e Adequate margin of exposure for the flavouring agent or related substances?	Follow-on from step B3 Are additional data available for the fla- vouring agent with an estimated dietary exposure exceeding the threshold of concern?	Comments on predicted metabo- lism applicable)	Conclusion based on current estimated dietary exposure
Flavouring agent	No.	CAS No. and structure					
Structural class I							
Ethyl alpha- acetyl/cinnamate	2211	620-80-4 	NR	NR	NA	Note 1	No safety concern
Ethyl 2-hydroxy-3- phenylpropionate	2213	15399-05-0 	NR	NR	NA	Note 2	No safety concern
Structural class III							
3-(3,4- Methylenedioxyphenyl)- 2-methylpropanal	2212	1205-17-0 	NA	NA	Yes. Some data are available; however, an appropriate repeated-dose toxicity study on No. 2212 or a closely related substance is not available.	Note 3	Additional data required to complete evaluation

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

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

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

Notes:

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

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

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

The aromatic acetals cinnamaldehyde propyleneglycol acetal (No. 2214) and 2-phenylpropanal propyleneglycol acetal (No. 2215) are expected to readily hydrolyse, yielding the corresponding aldehydes and 1,2-propanediol. Following oxidation to their corresponding acids, 2-phenylpropionic acid is excreted in the urine as the glucuronic acid conjugate, and cinnamic acid is excreted in the urine as hippuric acid following beta-oxidation to benzoic acid and conjugation with glycine. 1,2-Propanediol is metabolized to lactic acid and pyruvic acid.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the additional flavouring agents, the Committee assigned two flavouring agents (Nos 2211 and 2213) to structural class I and three flavouring agents (Nos 2212, 2214 and 2215) to structural class III [4].

Step 2. Four of the flavouring agents (Nos 2211, 2213, 2214 and 2215) in this group are predicted to be metabolized to innocuous products. The evaluation of these flavouring agents therefore proceeded via the A-side of the Procedure. The other flavouring agent (No. 2212) in this group cannot be predicted to be metabolized to innocuous products. Therefore, the evaluation of this flavouring agent proceeded via the B-side of the Procedure.

Step A3. The highest estimated dietary exposures for each of the two flavouring agents in structural class I that are predicted to be metabolized to innocuous products (Nos 2211 and 2213) are below the threshold of concern (i.e. 1800 µg/day for class I). The Committee therefore concluded that neither of the

two flavouring agents would pose a safety concern at current estimated dietary exposures. The two flavouring agents in structural class III that are predicted to be metabolized to innocuous products (Nos 2214 and 2215) have estimated dietary exposures greater than the threshold of concern (i.e. 90 µg/day for class III). Accordingly, the evaluation of these flavouring agents proceeded to step A4.

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

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

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

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

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

In accordance with the Procedure, additional data were evaluated for 3-(3,4-methylenedioxyphenyl)-2-methylpropanal (No. 2212), as its estimated dietary exposure exceeded the threshold of concern for structural class III (90 µg/day). Studies of acute toxicity, genotoxicity, and reproductive and developmental toxicity were available. Oral LD₅₀ values in mice and rats were reported as 1035 and 3561 mg/kg bw, respectively [6, 7]. Reverse mutation assays in *Salmonella typhimurium* and *Escherichia coli*, with and without exogenous metabolic activation, were negative [8]. An in vitro chromosome aberration assay in Chinese hamster ovary cells was positive for structural chromosome aberrations

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

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

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

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

Consideration of combined intakes from use as flavouring agents

The five additional flavouring agents in this group of cinnamyl alcohol and related substances have low MSDI values (0.01–4 µg/day). The Committee concluded that consideration of combined intakes is not necessary, because the additional flavouring agents would not contribute significantly to the combined intake of this group.

Consideration of secondary components

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

Consideration of additional data on previously evaluated flavouring agents

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

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

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

Conclusions

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

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

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

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

An addendum to the monograph was prepared.

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4.1.5 Tetrahydrofuran and furanone derivatives

Introduction

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

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

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

Assessment of dietary exposure

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

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

Absorption, distribution, metabolism and elimination

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

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

The alkyl-substituted furanone derivatives (Nos 2230, 2232 and 2234) need to undergo ring or side-chain oxidation, or keto-reduction by cytosolic carbonyl reductase, before conjugation of the resulting alcohol and excretion in the urine.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the above-mentioned flavouring agents, the Committee assigned one flavouring agent (No. 2230) to structural class II and four flavouring agents (Nos 2231–2234) to structural class III [5].

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

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

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

Table 5
Summary of the results of the safety evaluations of tetrahydrofuran and furanone derivatives used as flavouring agents^{a,b,c}

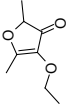
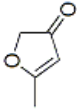
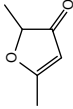
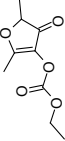
Flavouring agent	No.	CAS No. and structure	Step A3/B3 ^d Does estimated dietary exposure exceed the threshold of concern?	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 ^e Adequate margin of exposure for the flavouring agent or related substances?	Follow-on from step B3 ^e Are additional data available for the flavouring agent with an estimated dietary exposure exceeding the threshold of concern?	Comments on predicted metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
Structural class II									
2,5-Dimethyl-3(2H)-furanone	2230	14400-67-0 	B3: Yes, SPET: 600	NR	NR	Yes. No. 2230 is non-genotoxic in bacteria and in mammalian cells in vitro, and the NOAEL of 15 mg/kg bw per day in a 90-day study in rats [9] is 1 500 times the estimated dietary exposure to No. 2230 when used as a flavouring agent.	Note 1	–	No safety concern
Structural class III									
2,5-Dimethyl-4-ethoxy-3(2H)-furanone	2231	65330-49-6 	A3: Yes, SPET: 600	No	Yes. The NOAEL of 200 mg/kg bw per day for the structurally related 4-hydroxy-2,5-dimethyl-3(2H)-furanone (No. 1446) in a 2-year study in rats [6] is 20 000 times the estimated dietary exposure to No. 2231 when used as a flavouring agent.	NR	Note 2	4-Hydroxy-2,5-dimethyl-3(2H)-furanone (No. 1446) 	No safety concern
5-Methyl-3(2H)-furanone	2232	35111-32-8 	B3: Yes, SPET: 600	NR	NR	Yes, for the structurally related 2,5-dimethyl-3(2H)-furanone (No. 2230). This compound is non-genotoxic in bacteria and in mammalian cells in vitro, and the NOAEL of 15 mg/kg bw per day in a 90-day study in rats [9] is 1 500 times the estimated dietary exposure to No. 2232 when used as a flavouring agent.	Note 1	2,5-Dimethyl-3(2H)-furanone (No. 2230) 	No safety concern

Table 5 (continued)

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

NR: not relevant

^a Eighteen flavouring agents in this group were previously evaluated by the Committee (Annex 1, reference 173).

^b Step 1: One flavouring agent (No. 2230) is in structural Class II, and four flavouring agents (Nos 2231–2234) are in structural Class III.

^c Step 2: Nos 2231 and 2233 can be predicted to be metabolized to innocuous products. Nos 2230, 2232 and 2234 cannot be predicted to be metabolized to innocuous products.

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

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

Notes:

1. Ring or side-chain hydroxylation followed by conjugation with glucuronic acid and excretion in the urine.
2. The ether group is readily oxidized, and the resulting furanone is conjugated with glucuronic acid and excreted in the urine.
3. The carbonate group is readily hydrolysed, and the resulting furanone is conjugated with glucuronic acid and excreted in the urine.
4. Keto-reduction by cytosolic carbonyl reductase, followed by conjugation with glucuronic acid and excretion in the urine.

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

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

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

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

In accordance with the Procedure, additional data were evaluated for Nos 2230, 2232 and 2234, as their estimated dietary exposures exceeded the threshold of concern for structural class II (540 µg/day; No. 2230) or structural class III (90 µg/day; Nos 2232 and 2234).

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

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

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

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

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

Consideration of combined intakes from use as flavouring agents

The five additional flavouring agents in the group of tetrahydrofuran and furanone derivatives have low MSDIs (0.01–6 µg/day). The Committee concluded that consideration of combined intakes is not necessary, because the additional flavouring agents would not contribute significantly to the combined intake of this flavouring group.

Consideration of secondary components

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

Conclusion

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

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

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

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

An addendum to the monograph was prepared.

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4.2 Specifications of identity and purity of flavouring agents

4.2.1 New specifications

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

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

4.2.2 Revised specifications

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

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

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

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

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

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

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

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

5. Future work and recommendations

General considerations

Revision of the Procedure for the Safety Evaluation of Flavouring Agents

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

Approach for prioritizing flavouring agents for re-evaluation

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

Replacement of packed column gas chromatographic methods in the specifications monographs

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

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

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

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

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

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

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

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

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

General inclusion of infrared spectra

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

Inclusion of chemical structures in the JECFA flavourings database

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

Specific food additives (other than flavouring agents)***Carob bean gum***

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

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

Cassia gum

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

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

Citric and fatty acid esters of glycerol (CITREM)

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

Lutein esters from *Tagetes erecta*

The Committee at its seventy-ninth meeting considered establishing a group ADI “not specified” for lutein esters from *Tagetes erecta* that would include lutein from *Tagetes erecta* and synthetic zeaxanthin and related xanthophylls. The current Committee was not able to consider this aspect in detail and recommended that this be taken up at a future meeting.

Modified starches

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

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

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

Rosemary extract

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

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

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

Steviol glycosides

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

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

Flavouring agents

Aliphatic and aromatic amines and amides

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

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

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

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

Additional toxicological and/or dietary exposure information is required to complete the toxicological evaluation of six flavouring agents (Nos 2005, 2010, 2011, 2212, 2229 and 2234).

Acknowledgements

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

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

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Annex 1

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

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Annex 2

Toxicological information and information on specifications

Food additives evaluated toxicologically and assessed for dietary exposure

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

(continued)

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

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

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

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

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

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

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

^e ADI “not specified” is used to refer to a food substance of very low toxicity that, on the basis of the available data (chemical, biochemical, toxicological and other) and the total dietary exposure to the substance arising from its use at the levels necessary to achieve the desired effects and from its acceptable background levels in food, does not, in the opinion of the Committee, represent a hazard to health. For that reason, and for the reasons stated in the individual evaluations, the establishment of an ADI expressed in numerical form is not deemed necessary. An additive meeting this criterion must be used within the bounds of good manufacturing practice – i.e. it should be technologically efficacious and should be used at the lowest level necessary to achieve this effect, it should not conceal food of inferior quality or adulterated food, and it should not create a nutritional imbalance.

^f The tentative status was removed.

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

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

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

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

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

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

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

Food additives considered for specifications only

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

R: existing specifications revised; T: tentative specifications

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

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

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

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

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

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

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

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

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

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

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

N: new specifications

B. Aliphatic and aromatic amines and amides

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

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

M: existing specifications maintained; N: new specifications

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

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

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

C. Aliphatic secondary alcohols, ketones and related esters

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

N: new specifications

D. Cinnamyl alcohol and related substances

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

N: new specifications

^a The specifications include a statement that the safety evaluation for the flavouring agent had not been completed.**E. Tetrahydrofuran and furanone derivatives**

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

N: new specifications

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

Flavouring agents considered for specifications only

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

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

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

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

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

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

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

Annex 3

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

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

Annex 4

Meeting agenda



Food and Agriculture
Organization of the
United Nations



World Health
Organization

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

Opening:
Room C, 7 June at 9.30h

Draft Agenda

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

Food Additives

- 7.1 Toxicological Evaluation, Exposure Assessment, and Establishment of Specifications:
 - *Acacia polyacantha* var. *Campylacantha*, kakamut gum, arabino-galactan protein complex
 - Allura Red AC (INS 129)
 - Carob bean gum (INS 410)
 - Octenyl succinic acid modified gum arabic (INS 423)
 - Pectin

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

7.2 Food additives for revision of specifications and analytical methods:

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

Flavourings

7.3 Toxicological evaluation, exposure assessment and establishment of specifications for certain flavourings

- Cinnamyl alcohol and related substances
- Aliphatic secondary alcohols, ketones and related esters
- Alicyclic, alicyclic-fused and aromatic-fused ring lactones
- Aliphatic and aromatic amines and amides
- Tetrahydrofuran and furanone derivatives

8. Revision of specifications for certain flavourings

9. Other matters to be considered (general considerations)

For discussion: Proposal for revised decision tree for the evaluation of flavours

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

11. Adoption of the report.

SELECTED WHO PUBLICATIONS OF RELATED INTEREST

Evaluation of Certain Veterinary Drug Residues in Food

Eighty-first Report of the Joint FAO/WHO Expert Committee on Food Additives

WHO Technical Report Series, No. 997, 2016 (110 pages)

Toxicological Evaluation of Certain Veterinary Drug Residues in Food

Eighty-first Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)

WHO Food Additives Series, No. 72, 2016 (162 pages)

Evaluation of Certain Food Additives and Contaminants

Eightieth Report of the Joint FAO/WHO Expert Committee on Food Additives

WHO Technical Report Series, No. 995, 2016 (114 pages)

Safety Evaluation of Certain Food Additives and Contaminants

Eightieth Meeting of the Joint FAO/WHO Expert Committee on Food Additives

WHO Food Additives Series, No. 71, 2015 (132 pages)

Evaluation of Certain Food Additives

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

WHO Technical Report Series, No. 990, 2015 (124 pages)

Safety Evaluation of Certain Food Additives

Seventy-ninth Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)

WHO Food Additives Series, No. 70, 2015 (369 pages)

Evaluation of Certain Veterinary Drug Residues in Food

Seventy-eighth Report of the Joint FAO/WHO Expert Committee on Food Additives

WHO Technical Report Series, No. 988, 2014 (127 pages)

Toxicological Evaluation of Certain Veterinary Drug Residues in Food

Seventy-eighth Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)

WHO Food Additives Series, No. 69, 2014 (241 pages)

Evaluation of Certain Food Additives and Contaminants

Seventy-seventh Report of the Joint FAO/WHO Expert Committee on Food Additives

WHO Technical Report Series, No. 983, 2013 (75 pages)

Safety Evaluation of Certain Food Additives and Contaminants

Seventy-seventh Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)

WHO Food Additives Series, No. 68, 2013 (335 pages)

Evaluation of certain food additives

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

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

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

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

ISBN 978 92 4 121000 3



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Pregel™ Adhere 2000

Safety Data Sheet

According To Federal Register / Vol. 77, No. 58 / Monday, March 26, 2012 / Rules And Regulations And According To The Hazardous Products Regulation (February 11, 2015).

Revision Date: 12/18/2020

Date of Issue: 05/26/2015

Supersedes Date: 08/31/2011

Version: 2.1

SECTION 1: IDENTIFICATION

1.1. Product Identifier

Product Form: Substance

Product Name: Pregel™ Adhere 2000

Chemical Name: Wheat Starch, Modified: Oxidized Starch (INS No. 1404)

CAS-No.: 65996-62-5

1.2. Intended Use of the Product

For professional use only.

1.3. Name, Address, and Telephone of the Responsible Party

Manufacturer

MGP Ingredients, Inc.®

Cray Business Plaza, 100 Commercial Street

Atchison, KS 66002, USA

T (913) 367-1480

www.mgpingredients.com

1.4. Emergency Telephone Number

Emergency Number : 1-800-424-9300

CHEMTREC – TOLL FREE 24 HOUR EMERGENCY TELEPHONE NUMBER

SECTION 2: HAZARDS IDENTIFICATION

2.1. Classification of the Substance or Mixture

GHS-US/CA Classification

Comb. Dust

Full text of hazard classes and H-statements : see section 16

2.2. Label Elements

GHS-US/CA Labeling

Signal Word (GHS-US/CA)

: Warning

Hazard Statements (GHS-US/CA)

: May form combustible dust concentrations in air.

Supplemental Information

: Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. Proper grounding procedures to avoid static electricity should be followed. Prevent dust accumulation (to minimize explosion hazard). Avoid generating dust.

2.3. Other Hazards

No data available

2.4. Unknown Acute Toxicity (GHS-US/CA)

No data available

SECTION 3: COMPOSITION/INFORMATION ON INGREDIENTS

3.1. Substance

Name : Pregel™ Adhere 2000

CAS-No. : 65996-62-5

Name	Product Identifier	% *	GHS Ingredient Classification
Starch, oxidized	(CAS-No.) 65996-62-5	100	Comb. Dust

Full text of H-phrases: see section 16

3.2. Mixture

Not applicable

*Percentages are listed in weight by weight percentage (w/w%) for liquid and solid ingredients. Gas ingredients are listed in volume by volume percentage (v/v%).

SECTION 4: FIRST AID MEASURES

4.1. Description of First-aid Measures

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According To Federal Register / Vol. 77, No. 58 / Monday, March 26, 2012 / Rules And Regulations And According To The Hazardous Products Regulation (February 11, 2015).

General: Never give anything by mouth to an unconscious person. If you feel unwell, seek medical advice (show the label where possible).

Inhalation: Using proper respiratory protection, move the exposed person to fresh air at once. Encourage exposed person to cough, spit out, and blow nose to remove dust. Immediately call a poison center, physician, or emergency medical service.

Skin Contact: Remove contaminated clothing. Wash with plenty of soap and water. Drench affected area with water for at least 5 minutes. Obtain medical attention if irritation develops or persists.

Eye Contact: Rinse cautiously with water for at least 5 minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Obtain medical attention if irritation develops or persists.

Ingestion: Rinse mouth. Do NOT induce vomiting. Obtain medical attention.

4.2. Most Important Symptoms and Effects Both Acute and Delayed

General: Dust may cause mechanical irritation to eyes, nose, throat, and lungs. May cause an allergic reaction in sensitive individuals. Not expected to present a significant hazard under anticipated conditions of normal use.

Inhalation: Dust from this product may cause irritation to the respiratory tract. Dust may be harmful or cause irritation.

Skin Contact: Prolonged contact with large amounts of dust may cause mechanical irritation. Dust may cause irritation in skin folds or by contact in combination with tight clothing. Prolonged exposure may cause skin irritation.

Eye Contact: Prolonged contact with large amounts of dust may cause mechanical irritation. May cause slight irritation to eyes.

Ingestion: Ingestion may cause adverse effects.

Chronic Symptoms: None known.

4.3. Indication of Any Immediate Medical Attention and Special Treatment Needed

If medical advice is needed, have product container or label at hand. If exposed or concerned, get medical advice and attention.

SECTION 5: FIRE-FIGHTING MEASURES

5.1. Extinguishing Media

Suitable Extinguishing Media: Use extinguishing media appropriate for surrounding fire. Water spray, fog, carbon dioxide (CO₂), alcohol-resistant foam, or dry chemical.

Unsuitable Extinguishing Media: Do not use water jet. Use of heavy stream of water may spread fire. Do not use a heavy water stream.

5.2. Special Hazards Arising From the Substance or Mixture

Fire Hazard: Combustible Dust.

Explosion Hazard: Product itself is not explosive but if dust is generated, dust clouds suspended in air can be explosive. Dust explosion hazard in air.

Reactivity: Hazardous reactions will not occur under normal conditions.

5.3. Advice for Firefighters

Precautionary Measures Fire: Exercise caution when fighting any chemical fire. Under fire conditions, hazardous fumes will be present.

Firefighting Instructions: Avoid dust clouds in combination with static electricity. Use water spray or fog for cooling exposed containers.

Protection During Firefighting: Do not enter fire area without proper protective equipment, including respiratory protection.

Hazardous Combustion Products: Carbon oxides (CO, CO₂).

Other Information: Risk of dust explosion.

5.4. Reference to Other Sections

Refer to Section 9 for flammability properties.

SECTION 6: ACCIDENTAL RELEASE MEASURES

6.1. Personal Precautions, Protective Equipment and Emergency Procedures

General Measures: Avoid generating dust. Avoid all unnecessary exposure. Avoid breathing dust. Keep away from open flames, hot surfaces and sources of ignition. Use only non-sparking tools. Avoid prolonged contact with eyes, skin and clothing. Remove ignition sources. Keep away from heat, hot surfaces, sparks, open flames, and other ignition sources. No smoking.

6.1.1. For Non-Emergency Personnel

Protective Equipment: Use appropriate personal protective equipment (PPE).

Emergency Procedures: Evacuate unnecessary personnel.

6.1.2. For Emergency Personnel

Protective Equipment: Equip cleanup crew with proper protection.

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Safety Data Sheet

According To Federal Register / Vol. 77, No. 58 / Monday, March 26, 2012 / Rules And Regulations And According To The Hazardous Products Regulation (February 11, 2015).

Emergency Procedures: Upon arrival at the scene, a first responder is expected to recognize the presence of dangerous goods, protect oneself and the public, secure the area, and call for the assistance of trained personnel as soon as conditions permit. Ventilate area.

6.2. Environmental Precautions

Prevent entry to sewers and public waters.

6.3. Methods and Materials for Containment and Cleaning Up

For Containment: Contain and collect as any solid. Avoid generation of dust during clean-up of spills.

Methods for Cleaning Up: Clean up spills immediately and dispose of waste safely. Vacuum clean-up is preferred. If sweeping is required use a dust suppressant. Use explosion proof vacuum during cleanup, with appropriate filter. Do not mix with other materials. Vacuum must be fitted with HEPA filter to prevent release of particulates during clean-up. Use only non-sparking tools. Avoid generation of dust during clean-up of spills. Contact competent authorities after a spill.

6.4. Reference to Other Sections

See Section 13, Disposal Considerations. See Section 8 for exposure controls and personal protection and Section 13 for disposal considerations.

SECTION 7: HANDLING AND STORAGE

7.1. Precautions for Safe Handling

Additional Hazards When Processed: May form combustible dust concentrations in air. Good housekeeping is needed during storage, transfer, handling, and use of this material to avoid excessive dust accumulation. Keep away from heat, sparks, open flames, hot surfaces. – No smoking. Accumulation and dispersion of dust with an ignition source can cause a combustible dust explosion. Keep dust levels to a minimum and follow applicable regulations.

Precautions for Safe Handling: Wash hands and other exposed areas with mild soap and water before eating, drinking or smoking and when leaving work. Avoid prolonged contact with eyes, skin and clothing. Avoid creating or spreading dust. Keep away from heat, sparks, open flames, and hot surfaces. No smoking.

Hygiene Measures: Handle in accordance with good industrial hygiene and safety procedures.

7.2. Conditions for Safe Storage, Including Any Incompatibilities

Technical Measures: Avoid creating or spreading dust. Comply with applicable regulations. Use explosion-proof electrical, ventilating, lighting equipment. Proper grounding procedures to avoid static electricity should be followed.

Storage Conditions: Store in a dry, cool and well-ventilated place. Keep container closed when not in use. Protect from moisture. Keep/Store away from direct sunlight, extremely high or low temperatures and incompatible materials.

Incompatible Materials: Strong acids, strong bases, strong oxidizers.

7.3. Specific End Use(s)

For professional use only.

SECTION 8: EXPOSURE CONTROLS/PERSONAL PROTECTION

8.1. Control Parameters

For substances listed in section 3 that are not listed here, there are no established exposure limits from the manufacturer, supplier, importer, or the appropriate advisory agency including: ACGIH (TLV), AIHA (WEEL), NIOSH (REL), OSHA (PEL), or Canadian provincial governments.

8.2. Exposure Controls

Appropriate Engineering Controls: Ensure all national/local regulations are observed. Suitable eye/body wash equipment should be available in the vicinity of any potential exposure. Ensure adequate ventilation, especially in confined areas. Proper grounding procedures to avoid static electricity should be followed. Use explosion-proof equipment. Use local exhaust or general dilution ventilation or other suppression methods to maintain dust levels below exposure limits. Power equipment should be equipped with proper dust collection devices. It is recommended that all dust control equipment such as local exhaust ventilation and material transport systems involved in handling of this product contain explosion relief vents or an explosion suppression system or an oxygen-deficient environment.

Personal Protective Equipment: Insufficient ventilation: wear respiratory protection. Protective goggles. Gloves. Protective clothing.



Materials for Protective Clothing: Chemically resistant materials and fabrics.

Hand Protection: In case of repeated or prolonged contact wear gloves.

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Safety Data Sheet

According To Federal Register / Vol. 77, No. 58 / Monday, March 26, 2012 / Rules And Regulations And According To The Hazardous Products Regulation (February 11, 2015).

Eye and Face Protection: Chemical safety goggles.

Skin and Body Protection: Wear suitable protective clothing.

Respiratory Protection: If exposure limits are exceeded or irritation is experienced, approved respiratory protection should be worn. In case of inadequate ventilation, oxygen deficient atmosphere, or where exposure levels are not known wear approved respiratory protection.

Other Information: When using, do not eat, drink or smoke.

SECTION 9: PHYSICAL AND CHEMICAL PROPERTIES

9.1. Information on Basic Physical and Chemical Properties

Physical State	: Not available
Appearance	: White
Odor	: Bland
Odor Threshold	: Not available
pH	: Not available
Evaporation Rate	: Not available
Melting Point	: Not available
Freezing Point	: Not available
Boiling Point	: Not available
Flash Point	: Not available
Auto-ignition Temperature	: Not available
Decomposition Temperature	: Not available
Flammability (solid, gas)	: Not available
Lower Flammable Limit	: 0.04 % g/L (oz/ft ³)
Upper Flammable Limit	: Not available
Vapor Pressure	: Not available
Relative Vapor Density at 20°C	: Not available
Relative Density	: Not available
Specific Gravity	: ≈ 1.5
Solubility	: Negligible.
Partition Coefficient: N-Octanol/Water	: Not available
Viscosity	: Not available

SECTION 10: STABILITY AND REACTIVITY

10.1. Reactivity: Hazardous reactions will not occur under normal conditions.

10.2. Chemical Stability: Product itself is not explosive but if dust is generated, dust clouds suspended in air can be explosive. Stable under recommended handling and storage conditions (see section 7).

10.3. Possibility of Hazardous Reactions: Hazardous polymerization will not occur.

10.4. Conditions to Avoid: Avoid creating or spreading dust. Sparks, heat, open flame and other sources of ignition. Direct sunlight, extremely high or low temperatures, and incompatible materials. Dust accumulation (to minimize explosion hazard).

10.5. Incompatible Materials: Strong acids, strong bases, strong oxidizers.

10.6. Hazardous Decomposition Products: Thermal decomposition generates: Carbon oxides (CO, CO₂).

SECTION 11: TOXICOLOGICAL INFORMATION

11.1. Information on Toxicological Effects - Product

Acute Toxicity (Oral): Not classified

Acute Toxicity (Dermal): Not classified

Acute Toxicity (Inhalation): Not classified

LD50 and LC50 Data: Not available

Skin Corrosion/Irritation: Not classified

Eye Damage/Irritation: Not classified

Respiratory or Skin Sensitization: Not classified

Germ Cell Mutagenicity: Not classified

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Carcinogenicity: Not classified

Specific Target Organ Toxicity (Repeated Exposure): Not classified

Reproductive Toxicity: Not classified

Specific Target Organ Toxicity (Single Exposure): Not classified

Aspiration Hazard: Not classified

Symptoms/Injuries After Inhalation: Dust from this product may cause irritation to the respiratory tract. Dust may be harmful or cause irritation.

Symptoms/Injuries After Skin Contact: Prolonged contact with large amounts of dust may cause mechanical irritation. Dust may cause irritation in skin folds or by contact in combination with tight clothing. Prolonged exposure may cause skin irritation.

Symptoms/Injuries After Eye Contact: Prolonged contact with large amounts of dust may cause mechanical irritation. May cause slight irritation to eyes.

Symptoms/Injuries After Ingestion: Ingestion may cause adverse effects.

Chronic Symptoms: None known.

11.2. Information on Toxicological Effects - Ingredient(s)

LD50 and LC50 Data: Not available

SECTION 12: ECOLOGICAL INFORMATION

12.1. Toxicity

Ecology - General: Not classified.

12.2. Persistence and Degradability

Pregel™ Adhere 2000 (65996-62-5)	
Persistence and Degradability	Not established.

12.3. Bioaccumulative Potential

Pregel™ Adhere 2000 (65996-62-5)	
Bioaccumulative Potential	Not established.

12.4. Mobility in Soil Not available

12.5. Other Adverse Effects

Other Information: Avoid release to the environment.

SECTION 13: DISPOSAL CONSIDERATIONS

13.1. Waste treatment methods

Waste Disposal Recommendations: Dispose of contents/container in accordance with local, regional, national, territorial, provincial, and international regulations.

Ecology - Waste Materials: Avoid release to the environment.

SECTION 14: TRANSPORT INFORMATION

The shipping description(s) stated herein were prepared in accordance with certain assumptions at the time the SDS was authored, and can vary based on a number of variables that may or may not have been known at the time the SDS was issued.

14.1. In Accordance with DOT Not regulated for transport

14.2. In Accordance with IMDG Not regulated for transport

14.3. In Accordance with IATA Not regulated for transport

14.4. In Accordance with TDG Not regulated for transport

SECTION 15: REGULATORY INFORMATION

15.1. US Federal Regulations

Pregel™ Adhere 2000 (65996-62-5)	
SARA Section 311/312 Hazard Classes	Physical hazard - Combustible dust
Starch, oxidized (65996-62-5)	
Listed on the United States TSCA (Toxic Substances Control Act) inventory	
EPA TSCA Regulatory Flag	XU - XU - indicates a substance exempt from reporting under the Chemical Data Reporting Rule, (40 CFR 711).

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15.2. US State Regulations

Neither this product nor its chemical components appear on any US state lists, or its chemical components are not required to be disclosed

15.3. Canadian Regulations

Starch, oxidized (65996-62-5)

Listed on the Canadian DSL (Domestic Substances List)

SECTION 16: OTHER INFORMATION, INCLUDING DATE OF PREPARATION OR LAST REVISION

Date of Preparation or Latest : 12/18/2020

Revision

Other Information : This document has been prepared in accordance with the SDS requirements of the OSHA Hazard Communication Standard 29 CFR 1910.1200 and Canada's Hazardous Products Regulations (HPR) SOR/2015-17.

GHS Full Text Phrases:

Comb. Dust	Combustible Dust

The information presented above is believed to be accurate and represents the best information currently available to MGP Ingredients, Inc.® (MGP). MGP makes no warranty, express or implied, with respect to such information, and assumes no liability resulting from its use. In no way shall MGP be liable for any claims, losses, or damages of any third party or for lost profits or any special, indirect, incidental, consequential, or exemplary damages even if MGP has been advised of the possibility of such damages.

NA GHS SDS 2015 (Can, US)

Safety data sheet Safety data sheet

acc. to Regulation (EC) No. 1907/2006 (REACH)



Starch , soluble

article number: **4701**

Version: **3.0 en**

Replaces version of: 2018-08-10

Version: (2)

date of compilation: 2016-11-04

Revision: 2022-06-03

SECTION 1: Identification of the substance/mixture and of the company/undertaking

1.1 Product identifier

Identification of the substance	Starch , soluble
Article number	4701
EC number	232-679-6
CAS number	65996-62-5

1.2 Relevant identified uses of the substance or mixture and uses advised against

Relevant identified uses:	Laboratory chemical Laboratory and analytical use
Uses advised against:	Do not use for products which come into contact with foodstuffs. Do not use for private purposes (household).

1.3 Details of the supplier of the safety data sheet

Carl Roth GmbH + Co KG
Schoemperlenstr. 3-5
D-76185 Karlsruhe
Germany

Telephone: +49 (0) 721 - 56 06 0

Telefax: +49 (0) 721 - 56 06 149

e-mail: sicherheit@carlroth.de

Website: www.carlroth.de

Competent person responsible for the safety data sheet: Department Health, Safety and Environment

e-mail (competent person): sicherheit@carlroth.de

1.4 Emergency telephone number

Name	Street	Postal code/city	Telephone	Website
National Poisons Information Service City Hospital	Dudley Rd	B187QH Birmingham	844 892 0111	

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

Classification acc. to GHS

This substance does not meet the criteria for classification.

2.2 Label elements

Labelling

not required

2.3 Other hazards

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acc. to Regulation (EC) No. 1907/2006 (REACH)



Starch , soluble

article number: **4701**

Dust explosion hazards.

Results of PBT and vPvB assessment

According to the results of its assessment, this substance is not a PBT or a vPvB.

SECTION 3: Composition/information on ingredients

3.1 Substances

Name of substance	Starch
Molecular formula	$(C_6H_{10}O_5)_n$
CAS No	65996-62-5
EC No	232-679-6

SECTION 4: First aid measures

4.1 Description of first aid measures



General notes

Take off contaminated clothing.

Following inhalation

Provide fresh air.

Following skin contact

Rinse skin with water/shower.

Following eye contact

Rinse cautiously with water for several minutes.

Following ingestion

Rinse mouth. Call a doctor if you feel unwell.

4.2 Most important symptoms and effects, both acute and delayed

Symptoms and effects are not known to date.

4.3 Indication of any immediate medical attention and special treatment needed

none

SECTION 5: Firefighting measures

5.1 Extinguishing media



Suitable extinguishing media

co-ordinate firefighting measures to the fire surroundings
water, foam, alcohol resistant foam, dry extinguishing powder, ABC-powder

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acc. to Regulation (EC) No. 1907/2006 (REACH)



Starch , soluble

article number: **4701**

Unsuitable extinguishing media

water jet

5.2 Special hazards arising from the substance or mixture

Combustible. Danger of dust explosion.

Hazardous combustion products

In case of fire may be liberated: Carbon monoxide (CO), Carbon dioxide (CO₂)

5.3 Advice for firefighters

In case of fire and/or explosion do not breathe fumes. Fight fire with normal precautions from a reasonable distance. Wear self-contained breathing apparatus.

SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures



For non-emergency personnel

Control of dust.

6.2 Environmental precautions

Keep away from drains, surface and ground water.

6.3 Methods and material for containment and cleaning up

Advice on how to contain a spill

Covering of drains. Take up mechanically.

Advice on how to clean up a spill

Take up mechanically.

Other information relating to spills and releases

Place in appropriate containers for disposal.

6.4 Reference to other sections

Hazardous combustion products: see section 5. Personal protective equipment: see section 8. Incompatible materials: see section 10. Disposal considerations: see section 13.

SECTION 7: Handling and storage

7.1 Precautions for safe handling

No special measures are necessary.

Advice on general occupational hygiene

Keep away from food, drink and animal feedingstuffs.

7.2 Conditions for safe storage, including any incompatibilities

Store in a dry place.

Incompatible substances or mixtures

Observe hints for combined storage.

Consideration of other advice:

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acc. to Regulation (EC) No. 1907/2006 (REACH)



Starch , soluble

article number: **4701**

Ventilation requirements

Use local and general ventilation.

Specific designs for storage rooms or vessels

Recommended storage temperature: 15 – 25 °C

7.3 Specific end use(s)

No information available.

SECTION 8: Exposure controls/personal protection

8.1 Control parameters

National limit values

Occupational exposure limit values (Workplace Exposure Limits)

Coun-try	Name of agent	CAS No	Identifi-er	TWA [mg/m ³]	STEL [mg/m ³]	Ceil-ing-C [mg/m ³]	Nota-tion	Source
GB	dust		WEL	10			i	EH40/2005
GB	dust		WEL	4			r	EH40/2005

Notation

Ceiling-C Ceiling value is a limit value above which exposure should not occur

i Inhalable fraction

r Respirable fraction

STEL Short-term exposure limit: a limit value above which exposure should not occur and which is related to a 15-minute period (unless otherwise specified)

TWA Time-weighted average (long-term exposure limit); measured or calculated in relation to a reference period of 8 hours time-weighted average (unless otherwise specified)

8.2 Exposure controls

Individual protection measures (personal protective equipment)

Eye/face protection



Use safety goggle with side protection.

Skin protection



• hand protection

Wear suitable gloves. Chemical protection gloves are suitable, which are tested according to EN 374.

• type of material

NBR (Nitrile rubber)

• material thickness

>0,11 mm

• breakthrough times of the glove material

>480 minutes (permeation: level 6)

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acc. to Regulation (EC) No. 1907/2006 (REACH)



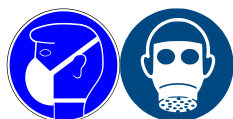
Starch , soluble

article number: **4701**

• other protection measures

Take recovery periods for skin regeneration. Preventive skin protection (barrier creams/ointments) is recommended.

Respiratory protection



Respiratory protection necessary at: Dust formation. Particulate filter device (EN 143). P1 (filters at least 80 % of airborne particles, colour code: White).

Environmental exposure controls

Keep away from drains, surface and ground water.

SECTION 9: Physical and chemical properties

9.1 Information on basic physical and chemical properties

Physical state	solid
Form	powder
Colour	white - light yellow
Odour	odourless
Melting point/freezing point	not determined
Boiling point or initial boiling point and boiling range	not determined
Flammability	this material is combustible, but will not ignite readily
Lower and upper explosion limit	not determined
Flash point	not applicable
Auto-ignition temperature	>400 °C
Decomposition temperature	not relevant
pH (value)	5,5 – 7,5
Kinematic viscosity	not relevant
<u>Solubility(ies)</u>	
Water solubility	~350 g/l at 90 °C
<u>Partition coefficient</u>	
Partition coefficient n-octanol/water (log value):	this information is not available
Vapour pressure	not determined
<u>Density and/or relative density</u>	
Density	not determined

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acc. to Regulation (EC) No. 1907/2006 (REACH)



Starch , soluble

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Relative vapour density	information on this property is not available

Particle characteristics	No data available.

Other safety parameters

Oxidising properties	none

9.2 Other information

Information with regard to physical hazard classes:	hazard classes acc. to GHS (physical hazards): not relevant

Other safety characteristics:

Dust explosion class	ST 1 Weak explosive (rate of pressure; Kst > 0 - < 200 bar m/s)

SECTION 10: Stability and reactivity

10.1 Reactivity

The product in the delivered form is not dust explosion capable; the enrichment of fine dust however leads to the danger of dust explosion.

10.2 Chemical stability

The material is stable under normal ambient and anticipated storage and handling conditions of temperature and pressure.

10.3 Possibility of hazardous reactions

Violent reaction with: strong oxidiser

10.4 Conditions to avoid

Keep away from heat.

10.5 Incompatible materials

There is no additional information.

10.6 Hazardous decomposition products

Hazardous combustion products: see section 5.

SECTION 11: Toxicological information

11.1 Information on toxicological effects

Classification acc. to GHS

This substance does not meet the criteria for classification.

Acute toxicity

Shall not be classified as acutely toxic.

Skin corrosion/irritation

Shall not be classified as corrosive/irritant to skin.

Serious eye damage/eye irritation

Shall not be classified as seriously damaging to the eye or eye irritant.

Safety data sheet Safety data sheet

acc. to Regulation (EC) No. 1907/2006 (REACH)



Starch , soluble

article number: 4701

Respiratory or skin sensitisation

Shall not be classified as a respiratory or skin sensitiser.

Germ cell mutagenicity

Shall not be classified as germ cell mutagenic.

Carcinogenicity

Shall not be classified as carcinogenic.

Reproductive toxicity

Shall not be classified as a reproductive toxicant.

Specific target organ toxicity - single exposure

Shall not be classified as a specific target organ toxicant (single exposure).

Specific target organ toxicity - repeated exposure

Shall not be classified as a specific target organ toxicant (repeated exposure).

Aspiration hazard

Shall not be classified as presenting an aspiration hazard.

Symptoms related to the physical, chemical and toxicological characteristics

• If swallowed

Data are not available.

• If in eyes

Data are not available.

• If inhaled

Data are not available.

• If on skin

Data are not available.

• Other information

Health effects are not known.

11.2 Endocrine disrupting properties

Not listed.

11.3 Information on other hazards

There is no additional information.

SECTION 12: Ecological information

12.1 Toxicity

Shall not be classified as hazardous to the aquatic environment.

Biodegradation

Data are not available.

12.2 Process of degradability

Data are not available.

12.3 Bioaccumulative potential

Data are not available.

Safety data sheet Safety data sheet

acc. to Regulation (EC) No. 1907/2006 (REACH)



Starch , soluble

article number: **4701**

12.4 Mobility in soil

Data are not available.

12.5 Results of PBT and vPvB assessment

Data are not available.

12.6 Endocrine disrupting properties

Not listed.

12.7 Other adverse effects

Data are not available.

SECTION 13: Disposal considerations

13.1 Waste treatment methods



Consult the appropriate local waste disposal expert about waste disposal.

Sewage disposal-relevant information

Do not empty into drains.

13.2 Relevant provisions relating to waste

The allocation of waste identity numbers/waste descriptions must be carried out according to the EEC, specific to the industry and process. Waste catalogue ordinance (Germany).

13.3 Remarks

Waste shall be separated into the categories that can be handled separately by the local or national waste management facilities. Please consider the relevant national or regional provisions.

SECTION 14: Transport information

14.1 UN number or ID number	not subject to transport regulations
14.2 UN proper shipping name	not assigned
14.3 Transport hazard class(es)	none
14.4 Packing group	not assigned
14.5 Environmental hazards	non-environmentally hazardous acc. to the dangerous goods regulations
14.6 Special precautions for user	There is no additional information.
14.7 Maritime transport in bulk according to IMO instruments	The cargo is not intended to be carried in bulk.
14.8 Information for each of the UN Model Regulations	
Transport of dangerous goods by road, rail and inland waterway (ADR/RID/ADN) - Additional information	Not subject to ADR, RID and ADN.
International Maritime Dangerous Goods Code (IMDG) - Additional information	Not subject to IMDG.

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International Civil Aviation Organization (ICAO-IATA/DGR) - Additional information

Not subject to ICAO-IATA.

SECTION 15: Regulatory information

15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture

Relevant provisions of the European Union (EU)

Seveso Directive

2012/18/EU (Seveso III)			
No	Dangerous substance/hazard categories	Qualifying quantity (tonnes) for the application of lower and upper-tier requirements	Notes
	not assigned		

Deco-Paint Directive

VOC content	0 %

Industrial Emissions Directive (IED)

VOC content	0 %

Directive on the restriction of the use of certain hazardous substances in electrical and electronic equipment (RoHS)

not listed

Regulation concerning the establishment of a European Pollutant Release and Transfer Register (PRTR)

not listed

Water Framework Directive (WFD)

not listed

Regulation on the marketing and use of explosives precursors

not listed

Regulation on drug precursors

not listed

Regulation on substances that deplete the ozone layer (ODS)

not listed

Regulation concerning the export and import of hazardous chemicals (PIC)

not listed

Regulation on persistent organic pollutants (POP)

not listed

National regulations(GB)

List of substances subject to authorisation (GB REACH, Annex 14) / SVHC - candidate list

not listed

Restrictions according to GB REACH, Annex 17

not listed

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Other information

Directive 94/33/EC on the protection of young people at work. Observe employment restrictions under the Maternity Protection Directive (92/85/EEC) for expectant or nursing mothers.

National inventories

Country	Inventory	Status
AU	AIIC	substance is listed
CA	DSL	substance is listed
CN	IECSC	substance is listed
EU	ECSI	substance is listed
JP	CSCL-ENCS	substance is listed
KR	KECI	substance is listed
NZ	NZIoC	substance is listed
PH	PICCS	substance is listed
TR	CICR	substance is listed
TW	TCSI	substance is listed
US	TSCA	substance is listed

Legend

AIIC	Australian Inventory of Industrial Chemicals
CICR	Chemical Inventory and Control Regulation
CSCL-ENCS	List of Existing and New Chemical Substances (CSCL-ENCS)
DSL	Domestic Substances List (DSL)
ECSI	EC Substance Inventory (EINECS, ELINCS, NLP)
IECSC	Inventory of Existing Chemical Substances Produced or Imported in China
KECI	Korea Existing Chemicals Inventory
NZIoC	New Zealand Inventory of Chemicals
PICCS	Philippine Inventory of Chemicals and Chemical Substances (PICCS)
TCSI	Taiwan Chemical Substance Inventory
TSCA	Toxic Substance Control Act

15.2 Chemical Safety Assessment

No Chemical Safety Assessment has been carried out for this substance.

SECTION 16: Other information

Indication of changes (revised safety data sheet)

Alignment to regulation:

Restructuring: section 9, section 14

Section	Former entry (text/value)	Actual entry (text/value)	Safety-relevant
2.1	Classification according to Regulation (EC) No 1272/2008 (CLP): This substance does not meet the criteria for classification in accordance with Regulation No 1272/2008/EC.	Classification acc. to GHS: This substance does not meet the criteria for classification.	yes
2.2	Signal word: not required		yes
2.3	Other hazards: There is no additional information.	Other hazards: Dust explosion hazards.	yes

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Section	Former entry (text/value)	Actual entry (text/value)	Safety-relevant
2.3		Results of PBT and vPvB assessment: According to the results of its assessment, this substance is not a PBT or a vPvB.	yes

Abbreviations and acronyms

Abbr.	Descriptions of used abbreviations
ADN	Accord européen relatif au transport international des marchandises dangereuses par voies de navigation intérieures (European Agreement concerning the International Carriage of Dangerous Goods by Inland Waterways)
ADR	Accord relatif au transport international des marchandises dangereuses par route (Agreement concerning the International Carriage of Dangerous Goods by Road)
CAS	Chemical Abstracts Service (service that maintains the most comprehensive list of chemical substances)
Ceiling-C	Ceiling value
DGR	Dangerous Goods Regulations (see IATA/DGR)
EC No	The EC Inventory (EINECS, ELINCS and the NLP-list) is the source for the seven-digit EC number, an identifier of substances commercially available within the EU (European Union)
EH40/2005	EH40/2005 Workplace exposure limits (http://www.nationalarchives.gov.uk/doc/open-government-licence/)
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of Notified Chemical Substances
GB REACH	The REACH etc. (Amendment etc.) (EU Exit) Regulations 2019, SI 2019/758 (as amended)
GHS	"Globally Harmonized System of Classification and Labelling of Chemicals" developed by the United Nations
IATA	International Air Transport Association
IATA/DGR	Dangerous Goods Regulations (DGR) for the air transport (IATA)
ICAO	International Civil Aviation Organization
IMDG	International Maritime Dangerous Goods Code
NLP	No-Longer Polymer
PBT	Persistent, Bioaccumulative and Toxic
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RID	Règlement concernant le transport International ferroviaire des marchandises Dangereuses (Regulations concerning the International carriage of Dangerous goods by Rail)
STEL	Short-term exposure limit
TWA	Time-weighted average
VOC	Volatile Organic Compounds
vPvB	Very Persistent and very Bioaccumulative
WEL	Workplace exposure limit

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Key literature references and sources for data

Agreement concerning the International Carriage of Dangerous Goods by Road (ADR). Regulations concerning the International Carriage of Dangerous Goods by Rail (RID). International Maritime Dangerous Goods Code (IMDG). Dangerous Goods Regulations (DGR) for the air transport (IATA).

Disclaimer

This information is based upon the present state of our knowledge. This SDS has been compiled and is solely intended for this product.