



Toxicological profile for Dextrin Sugars (dextrin)

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

(3R,4S,5S,6R)-2-[(2R,3S,4R,5R)-4,5-Dihydroxy-2-(hydroxymethyl)-6-[(2R,3S,4R,5R,6S)-4,5,6-trihydroxy-2-(hydroxymethyl)oxan-3-yl]oxyoxan-3-yl]oxyoxan-3-yl]oxy-6-(hydroxymethyl)oxane-3,4,5-triol (PubChem)

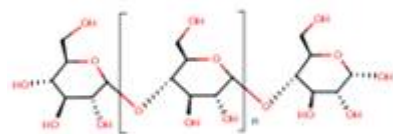
1.2. Synonyms

Dextrid; Dextrins; Fortodex; Corn dextrin; Dextrina Bianca; Dextrin 3; Caloreen; Dextrine; Nutriose; Eclipse G; Cream Dextrin 15; Dextrin 10; Dextrin 12; Dextrin 20; Farinex CO 2; EPA Pesticide Chemical Code 084503; (3R,4S,5S,6R)-2-[(2R,3S,4R,5R)-4,5-dihydroxy-2-(hydroxymethyl)-6-[(2R,3S,4R,5R,6S)-4,5,6-trihydroxy-2-(hydroxymethyl)oxan-3-yl]oxyoxan-3-yl]oxyoxan-3-yl]oxy-6-(hydroxymethyl)oxane-3,4,5-triol; Microcrystalline cellulose; Pyrodextrin; Deprexil; Dextrina; Fibersol-2; Indigestible dextrin; DEXTRIN, CORN; Dextrin (corn); Maltodextrin, dextrose equivalent 16-20; D-glucopyranosyl-(1->4)-D-glucopyranosyl-(1->4)-a-D-glucopyranose; tetrahydropyran-3-yl]oxy-tetrahydropyran-3-yl]oxy-6-ydroxymethyl) tetrahydropyran-3,4,5-triol; (3R,4S,5S,6R)-2-[[[(2R,3S,4R,5R)-4,5-dihydroxy-2-(hydroxymethyl)-6-[[[(2R,3S,4R,5R,6S)-4,5,6-trihydroxy-2-(hydroxymethyl)oxan-3-yl]oxy}oxan-3-yl]oxy}-6-(hydroxymethyl) oxane-3,4,5-triol; all-E)-9-(4-Methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-2,4,6,8-nonatetraenoicacidethylester; Dextrin, having more than 85% of its molecules with molecular masses between 1640 and 45,000 with a claimed-average molecular mass of approximately 20,000 (PubChem)

1.3. Molecular formula

C₁₈H₃₂O₁₆ (PubChem)

1.4. Structural Formula



1.5. Molecular weight (g/mol)

504.4 (PubChem)

1.6. CAS registration number

9004-53-9

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

“Soluble in water” (GESTIS)

1.7.4. pKa

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

No data available to us at this time.

1.7.10. Vapor pressure

No data available to us at this time.

1.7.11. log Kow

-6.9 (PubChem)

2. General information

2.1. Exposure

Used as binder, diluent, emulsion stabilizer, stiffening agent, thickening agent and viscosity increasing agent in non-medicinal natural health products.

As taken from Health Canada, 2021

“Used as an excipient for dry extracts and pills, to make emulsions and dry bandages, for thickening dye pastes and textile-dyeing mordants, sizing paper and fabrics, and printing tapestries. Also used in felt, printer's inks, glues, matches, fireworks and explosives. Used as a flavoring agent, anticaking agent, drying agent, formulation aid, humectant, lubricant or release agent, nutritive sweetener, solvent, and stabilizer or thickener for foods.

Industrial Processes with risk of exposure: Pulp and Paper Processing; Textiles (Fiber & Fabric Manufacturing); Textiles (Printing, Dyeing, or Finishing); Working with Glues and Adhesives

As taken from Haz-Map, 2020

INCI Name	DEXTRIN
Description	
CAS #	9004-53-9
EC #	232-675-4
Cosmetics Regulation provisions	
Functions	ABSORBENT, BINDING, BULKING, VISCOSITY CONTROLLING
SCCS opinions	
Identified INGREDIENTS or substances e.g.	

As taken from CosIng.

Dextrin is listed as a fragrance ingredient by IFRA.

Dextrin is listed as an ingredient (at concentrations, where specified) in home maintenance (<5-10%, includes “old” products), inside the home (10%, includes “old” products), “old” landscape/yard (10%) and personal care products by the CPID.

“Roquette provides an estimate of dietary exposure for enzyme-modified dextrans based on the intended use in sixteen food categories and using food consumption data from the National Health and Nutrition Examination Surveys conducted in 2003-2004 and 2005-2006. Based on the data, Roquette calculated a mean exposure of 17.4 grams/day (g/d) (0.3 g/kg body weight (bw)/d) and a 90th percentile of 32.6 g/d (0.6 g/kg bw/d). Roquette states that the use of enzyme-modified dextrans is limited by quality considerations and consumer acceptability.”

As taken from FDA, 2013.

2.2. Combustion products

This ingredient was investigated in a pyrolysis study. Results are given in Baker and Bishop (2005) J. Anal. Appl. Pyrolysis 74, pp. 145–170.

Ingredient Name & CAS Number	Max. cig. appln. level (ppm)	Purity of Sample (%)	Composition of pyrolysate (Compound, %)	Max. level in smoke (µg)
Dextrin 9004-53-9	1	na	Hydroxymethylfurfural (50.1)	0.3
			Furfural (23.5)	0.1
			Unidentified compound (4.8)	0.02
			Furfuryl alcohol? (4.2)	0.02
			Methyl resorcinol (1.8)	0.009

2.3. Ingredient(s) from which it originates

The term dextrin, in its broadest sense, may refer to any product obtained by any method (e.g., heat, acid, enzyme) for degrading the starch. The physical properties of dextrans can cover a very wide range. A synonym used for dextrans is starch gums. The tensile strength of dextrin film is lower than that for starch and decreases with the degree of conversion. However, dextrin formulations can be prepared at higher concentrations than unmodified starch yielding films with higher proportions of solids, which dry faster and are thicker.

As an example, yellow corn dextrin is used to encapsulate water-insoluble flavors and oils by spray-drying (Wurzburg 2006).

As taken from Encapsulation Technologies for Active Food Ingredients and Food Processing. Chapter 3. Materials for Encapsulation. Springer ScienceBusiness Media, LLC 2010. 978-1-4419-1008-0_3. Wandrey C et al., available https://books.google.co.uk/books?id=wi3GFjTn5gcC&printsec=copyright&redir_esc=y#v=onepage&q&f=false

“The subjects of the notice are two enzyme-modified dextrans, which differ by molecular weight (MW) distribution and average degree of polymerization....Roquette states that these two products are derived from yellow dextrin. Yellow dextrin with a MW of 3000-10,000 daltons is formed by heating corn or wheat starch in the presence of small amounts of food-grade acid. The yellow dextrin is partially hydrolyzed using food-grade alpha-amylase, to depolymerize readily digestible polysaccharides. To obtain the first product, the hydrolysate is treated with activated carbon for decolorization and cationic and anionic resins (regulated by FDA for such uses, 21 CFR 173.25) to remove impurities, and then spray dried. To obtain the second product, the purified hydrolysate is subjected to chromatographic separation to remove low molecular weight sugars and then spray dried. Roquette describes the average degree of polymerization of enzyme-modified dextrin as approximately 4-10 for the first product and 12-25 for the second product.”

As taken from FDA, 2013.

Found in ephedra and honey.

As taken from Khan IA and Abourashed EA, 2010.

3. Status in legislation and other official guidance

Dextrin is included on the US FDA's inventory of "Substances Added to Food (formerly EAFUS)" as an anticaking agent or free-flow agent, drying agent, flavor enhancer and a flavoring agent or adjuvant, formulation aid, humectant, lubricant or release agent, nutritive sweetener, solvent or vehicle, and a stabilizer or thickener, is included under 21 CFR section 172.892 (food starch-modified) and has GRAS status under section 184.1277 (FDA, 2022, 2023).

FDA PART 184 -- DIRECT FOOD SUBSTANCES AFFIRMED AS GENERALLY RECOGNIZED AS SAFE

Subpart B--Listing of Specific Substances Affirmed as GRAS

Sec. 184.1277 Dextrin.

(a) Dextrin ((C₆H₁₀O₅)_n.H₂O, CAS Reg. No. 9004-53-9) is an incompletely hydrolyzed starch. It is prepared by dry heating corn, waxy maize, waxy milo, potato, arrowroot, wheat, rice, tapioca, or sago starches, or by dry heating the starches after: (1) Treatment with safe and suitable alkalis, acids, or pH control agents and (2) drying the acid or alkali treated starch.

(b) The ingredient meets the specification of the Food Chemicals Codex, 3d Ed. (1981), p. 96, which is incorporated by reference. Copies are available from the National Academy Press, 2101 Constitution Ave. NW., Washington, DC 20418, or available for inspection at the National Archives and Records Administration (NARA). For information on the availability of this material at NARA, call 202-741-6030, or go to: http://www.archives.gov/federal_register/code_of_federal_regulations/ibr_locations.html.

(c) In accordance with 184.1(b)(1), the ingredient is used in food with no limitation other than current good manufacturing practice. The affirmation of this ingredient as generally recognized as safe (GRAS) as a direct human food ingredient is based upon the following current good manufacturing practice conditions of use:

(1) The ingredient is used as a formulation aid as defined in 170.3(o)(14) of this chapter; as a processing aid as defined in 170.3(o)(24) of this chapter; as a stabilizer and thickener as defined in 170.3(o)(28) of this chapter; and as a surface-finishing agent as defined in 170.3(o)(30) of this chapter.

(2) The ingredient is used in food at levels not to exceed current good manufacturing practice.

(d) Prior sanctions for this ingredient different from the uses established in this section do not exist or have been waived.

As taken from FDA, 2023.

"The subjects of the notice are two enzyme-modified dextrins, which differ by molecular weight (MW) distribution and average degree of polymerization. The notice informs FDA of the view of Roquette that enzyme-modified dextrins are GRAS, through scientific procedures, for use as bulking agents or as sources of fiber in baked goods, beverages (liquid non-dairy), cereals and granola bars, condiments and dressings, confections, dairy beverages, dairy (non-beverages), frozen desserts, gravies and sauces, meal replacements, pasta and grain products, prepared meals and soups (excluding meat and poultry products), processed fruits, shelf-stable desserts, snacks and crackers, and dry beverage powders, at levels of 3 to 9 grams per serving.

Based on the information provided by Roquette, and other information available to FDA, the agency has no questions at this time regarding Roquette's conclusion that the two enzyme-modified dextrins are GRAS under the intended conditions of use. The agency has not, however, made its own determination regarding the GRAS status of the subject use of enzyme-modified dextrins.

In accordance with proposed 21 CFR 170.36(f), a copy of the text of this letter responding to GRN 000436, as well as a copy of the information in this notice that conforms to the information in the GRAS exemption claim (proposed 21 CFR 170.36(c)(1)), is available for public review and copying at www.fda.gov/grasnoticeinventory."

As taken from FDA, 2013.

Dextrin (CAS RN 9004-53-9) is listed in the US EPA InertFinder Database (2023) as approved for food and non-food use pesticide products. For food use, it is regulated under 40 CFR Part 180.950e (Tolerances and Exemptions for Pesticide Chemical Residues in Food. Tolerance exemptions for minimal risk active and inert ingredients) (US EPA, 2023b).

Dextrin (CAS RN 9004-53-9) is not registered under REACH (ECHA).

Dextrin (CAS RN 9004-53-9) is not classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2023).

Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA):

DEXTRINS, WHITE AND YELLOW, ROASTED STARCH	
INS:	1400
Chemical names:	DEXTRINS
CAS number:	9004-53-9
Synonyms:	STARCH, ROASTED, WHITE AND YELLOW DEXTRINS
Functional class:	Food Additives STABILIZER; THICKENER
Evaluation year:	2018
Comments:	Considered for specification only.
Meeting:	86
Specs Code:	R, T
Report:	TRS 1014-JECFA 86/65
Tox Monograph:	FAO JECFA Monographs 22/53
Evaluation year:	2016
ADI:	NOT SPECIFIED
Meeting:	82
Specs Code:	R,T
Report:	TRS 1000-JECFA 82/83
Specification:	FAO JECFA Monographs 19/132
Evaluation year:	1982

ADI:	NOT SPECIFIED
Meeting:	26
Specs Code:	R (1989)
Report:	TRS 683-JECFA 26/29
Specifications:	FNP 49-JECFA 35/28 (1989); COMPENDIUM/965; FAO JECFA Monographs 1 vol.2/397 (MODIFIED STARCH)
Tox monograph:	FAS 17-JECFA 26/117
Previous Years:	1988, FNP 38-JECFA 33/142. R 1985, FNP 34-JECFA 29/137. R 1983, FNP 28-JECFA 27/76. R 1982, FNP 25-JECFA 26/121. R 1973, NMRS 53/TRS 539-JECFA 17/22, FNP 4-JECFA 17/60, FAS 5/NMRS 53A-JECFA 17/378. ADI NOT LIMITED. NL. R 1971, FAS 2/NMRS 50B-JECFA 15/45. N 1969, NMRS 46/TRS 445-JECFA 13/13, FAS 70.36/NMRS 46A-JECFA 13/63. ADI NOT LIMITED. NL. O

As taken from JECFA, 2021.

Dextrin is included on the US EPA's list of Safer Chemical Ingredients with functional use: processing aids and additives (US EPA, 2023a).

Dextrin is listed on the US EPA Toxic Substances Control Act (TSCA) inventory and also on the US EPA 2020 CDR and 2020 CDR Full Exempt lists (Chemical Data Reporting Rule).

US EPA 2020 CDR List. US EPA 2020 CDR Full Exempt List. US EPA TSCA inventory.

Dextrin is classified as a natural health product under Schedule 1, item 2 (an isolate) of the Natural Health Products Regulations (Health Canada, 2021).

Dextrin (CAS RN 9004-53-9) is listed by the US EPA Office of Pesticide Programs (undated) and was first registered as a "conventional chemical" pesticide on 1 July 1987.

Dextrin (CAS RN 9004-53-9) "poses no unreasonable risk to human health based on Tier I assessment under the NICNAS IMAP assessment framework" and has been "identified as low concern to human health by application of expert validated rules under the NICNAS targeted tier I approach" (AICIS, 2012).

4. Metabolism/Pharmacokinetics

4.1. Metabolism/metabolites

Dextrins and their parent starches were fed to groups of 6 weanling male rats (strain unspecified), initial weight 45-60 g, at a level of 60 g/kg bw for 21 to 28 days. Diets contained 18.8% casein. The digestibility of wheat dextrin was somewhat lower than that of wheat starch. The potato dextrin

gave a higher body weight gain and digestibility coefficient than the parent starch (Booher et al., 1951).

In a study on the effect of type of dietary carbohydrate on B-vitamin synthesis in the digestive tracts of rats, groups of 17 to 44 male and female rats (strain unspecified), 21 days of age were placed on test diets containing 18% protein (casein), 71% carbohydrate, and 3% butterfat, cod-liver oil and salt mixture. The carbohydrate sources used included cornstarch, corn dextrin, glucose, lactose and sucrose. Animals on all diets, without supplemental B-vitamins but with access to their faeces, showed low or declining growth rates after 2 weeks except for the group fed the dextrin diet.

Growth rates in all groups were increased after receiving faeces of the dextrin-fed group. Rats receiving the dextrin diet had enlarged caeca at the conclusion of the study. Caecectomized rats with access to their faeces lost weight when fed a dextrin diet; supplementation with baker's yeast resulted in weight gain. It was concluded that the peculiar property of corn dextrin was not due to retained B-vitamins, but rather to the formation of these vitamins in the lower part of the digestive tract of the rat as a result of incomplete digestion of this particular carbohydrate (Guerrant et al., 1935).

Fournier (1959) studied the effect of various dietary carbohydrate sources on calcium retention, serum calcium levels, and caecal size in the rat. Wistar rats (sex unspecified) weighing 62-74 g, were fed a low calcium diet (50 mg Ca/100 g diet) for 18 days after which groups of 6 rats were placed on diets containing 15% casein, 1.5% calcium carbonate and 45-70% experimental carbohydrate (starch, dextrin, caramel or glucose) plus cereal grain to bring total carbohydrate to about 70%. Rats received an estimate of 46 g dextrin per kg body weight. Calcium balance was determined during the third to fifth day; after 10 days the rats were sacrificed and serum calcium determined. Caecal enlargement observations were made after 2 weeks of feeding a diet containing 75% of the experimental carbohydrate source, 12% casein, 8% peanut oil and 3% salts. Calcium intake was approximately the same for all diets, but calcium retention for the dextrin and caramel diets was nearly double that for the starch and glucose diets. Serum calcium levels also were greater for the dextrin and caramel diets. Dry caecal weights of rats fed dextrin and caramel were more than double those fed the starch and glucose diets. The author postulated that dextrin and caramel were less easily metabolized than their parent substances, starch and glucose, and that this property contributed to the effects observed.

INCHEM (1982)

4.2. Absorption, distribution and excretion

¹⁴C-labelled beta-cyclodextrin homogenized in aqueous dextran was administered to 5 Wistar plus Long Evans F1 hybrid male rats weighing about 200 g each. Individual animals received doses corresponding to their body weight through an oesophageal cannula. Three control animals received ¹⁴C-labelled glucose. Blood levels of the compounds were measured at intervals up to 97 hours from tail vein samples. At 7, 12 and 24 hours after administration, selected animals were decapitated, and radioactivity was measured in stomach, small intestine and colon. In the case of the cyclodextrin, a maximum of only 5% of the administered activity could be found in blood even after 10 hours; after 96 hours the same residual radioactivity was found in the blood with both glucose and β -cyclodextrin. This study suggests that β -cyclodextrin cannot be absorbed either from the stomach or the small intestine; only the labelled open-chain dextrans and glucose formed from cyclodextrin by amylase action were absorbed (Szejtli et al., 1980). [i.e. Open-chain dextrans can be absorbed into the blood from the digestive system.]

INCHEM (1982)

"The characterization of biodistribution is a central requirement in the development of biomedical applications based on the use of nanoparticles, in particular for controlled drug delivery. The blood circulation time, organ biodistribution and rate of excretion must be well characterized in the

process of product development. In this work, the biodistribution of recently developed self-assembled dextrin nanoparticles is addressed. Functionalization of the dextrin nanoparticles with a DOTA-monoamide-type metal chelator, via click chemistry, is described. The metal chelator functionalized nanoparticles were labelled with a gamma-emitting (153)Sm(3+) radioisotope and the blood clearance rate and organ biodistribution of the nanoparticles were obtained. The effect of PEG surface coating on the blood clearance rate and organ biodistribution of the nanoparticles was also studied" (Goncalves et al. 2010a).

"The uptake of nanoparticles by cells of the mononuclear phagocytic system limits its use as colloidal drug carriers, reducing the blood circulation time and the ability to reach biological targets. In this work, the interaction between dextrin nanoparticles--recently developed in our laboratory--and murine bone marrow-derived macrophages was evaluated. FITC labelled nanoparticles were used to assess the phagocytic uptake and blood clearance after intravenous injection. The phagocytic uptake was analysed in vitro by confocal laser scanning microscopy and fluorescence activated cell sorting. Nanoparticles are phagocytosed by macrophages and are detected inside the cells, concentrated in cellular organelles. The blood clearance study showed that the blood removal of the nanoparticles occurs with a more pronounced rate in the first 3 h after intravenous administration, with about 30% of the material remaining in systemic circulation at this stage. Given the fairly high blood circulation time and biocompatibility, the dextrin nanoparticles are promising carriers for biomedical applications" (Goncalves et al. 2010b).

"....To date, all known and suspected prebiotics are carbohydrate compounds, primarily oligosaccharides, known to resist digestion in the human small intestine and reach the colon where they are fermented by the gut microflora. Studies have provided evidence that inulin and oligofructose (OF), lactulose, and resistant starch (RS) meet all aspects of the definition, including the stimulation of Bifidobacterium, a beneficial bacterial genus. Other isolated carbohydrates and carbohydrate-containing foods, including galactooligosaccharides (GOS), transgalactooligosaccharides (TOS), polydextrose, wheat dextrin, acacia gum, psyllium, banana, whole grain wheat, and whole grain corn also have prebiotic effects." As taken from Slavin J. 2013. Nutrients 5(4), 1417-35. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23609775>

4.3. Interactions

The interactions between dextrin and metal hydroxides in aqueous solutions (Abstract).

Dextrin, a common dispersing/depressing agent in flotation systems, was found to coprecipitate with metal hydroxides but not with metal cations in aqueous solutions. Such interactions caused a decrease in the solution pH. The optimum pH for dextrin-metal hydroxide coprecipitation was 7.5 for ferric, 8 for aluminum, 9 for cupric, 11 for lead, and 12 for magnesium ions. The pH ranges of the optimum coprecipitation coincide quite well with the IEP of the metal hydroxide. Infrared tests with the precipitates revealed that the reaction between dextrin and metal hydroxide eliminates the glucose ring deformation. Based on such observations it is concluded that the interactions between dextrin and metal hydroxide result from formation of chemical complexes and are not a result of hydrogen bonding. Qi Liu and J. S. Laskowski. Journal of Colloid and Interface Science, Volume 130, Issue 1, June 1989, Pages 101-111. As taken from ScienceDirect, 2011 available at www.sciencedirect.com

5. Toxicity

5.1. Single dose toxicity

"NUTRIOSE®FB10 is a dextrin considered a dietary fiber. The present study aims to assess the digestive tolerance of a high dose of NUTRIOSE®FB10 consumed over the day, and its effect on digestive symptoms. In a randomized, double-blind, cross-over trial, 12 healthy men ingested 1 l/day orange juice containing 50 g either NUTRIOSE®FB10 or placebo (maltodextrin) in three equal

doses at breakfast, lunch and 4:00 pm meal. Bloating, borborygmus, flatulence, nausea feelings, stomach ache, transit and stool consistency were evaluated at different times after the first consumption. Questionnaires on well-being and bowel movement were completed at 24 and 48 h. For all data except stool consistency, the area under the curve, the maximum score and the time of this maximum were calculated. For stool consistency, the mean score over 48 h was determined. There was no statistical difference between NUTRIOSE®FB10 and placebo on each criterion. NUTRIOSE®FB10 is well tolerated during a single day at 50 g divided into three doses” (Marteau et al. 2011).

Organism	Test Type	Route	Reported Dose (Normalized Dose)	Effect	Source
Mouse	LD50	Intravenous	350 mg(Fe)/kg		Toxicology and Applied Pharmacology. Vol. 23, Pg. 537, 1972.

As taken from PubChem

“Groups of 5 male and 5 female Sprague-Dawley rats weighing 150 to 160 g were administered cyclodextrins intravenously. The i.v. LD50 for rats was determined to be 0.79-1.0 g/kg bw with a close relationship between the acute toxicity and the nephrotoxic dose (Frank et al., 1976).”

INCHEM (1982)

5.2. Repeated dose toxicity

Groups of 10 male Wistar rats received diets with 6 or 15% protein from casein and 77 or 66% carbohydrate (75 and 65 g carbohydrate/kg bw). After 28 days, protein efficiency and weight gain/g of dry food were significantly lower for corn dextrin than for cornstarch, but were greater or equal to values for dextrose. Corn dextrin diets caused no unusual effect on liver weight or liver fat content; however, rats receiving corn dextrin exhibited a slight diarrhoea and caecal enlargement to about twice that in rats fed unmodified cornstarch (Reussner et al., 1963).

Groups of 6 Sprague-Dawley male weanling rats (initial weight 40-50 g), were fed for periods of 2-12 weeks on diets containing approximately 80 g dextrin/kg bw; diets contained 81% carbohydrate, 9% casein and 5% corn oil. Rates of gain with the dextrins over a 4-week period were about 15% less than that for autoclaved cornstarch; the latter weight was about double when the carbohydrate source was glucose or sucrose. Liver fat deposition was less for one of the dextrins, cornstarch or glucose, than for sucrose as the carbohydrate source. Liver fat deposition values were not reported for the other dextrin (Harper et al., 1953).

Groups of 5 or 10 male weanling Sprague-Dawley or Osborne-Mendel rats, weighing initially 40-50 g, were fed diets containing about 80 g carbohydrate/kg bw; diets consisted of 87% carbohydrate, 9% casein, 3% gelatin and 3% corn oil. Weight gain over a 4-week period with niacin supplementation was the same with either dextrin, starch or glucose as carbohydrate source, without niacin supplementation, growth rate decreased about 40% for starch and dextrin as the carbohydrate source as compared to 60% decrease with glucose as the carbohydrate source suggesting a lesser niacin requirement with starch and dextrin as carbohydrate source (Hundley, 1949).

Groups of 9 male Sprague-Dawley rats (2 months of age) were fed diets with different sources of carbohydrate for a 20-month period. Diets consisted of rat chow mixed with 20%, by weight, of the various carbohydrate sources, including dextrin, sucrose, and dextrose. Rats received approximately 10 g experimental carbohydrate/kg bw. Protein efficiency ratios calculated after 6 months feeding were nearly equal for the dextrin, dextrose and sucrose diets and significantly

higher than for the basal rat chow diet. Weight gain after 20 months of feeding was about 5% less for dextrin than for dextrose or sucrose but about 5% more than on the basal rat chow (Cohen et al., 1967).

INCHEM (1982)

“Roquette notes that the toxicological and human studies on enzyme-modified dextrin were performed using the product containing the higher fiber level. Roquette cites a published study that reported the results of...(a) 90 day Sprague-Dawley rat feeding study....The no observed adverse effect level from the 90 day rat study was the highest dose tested, which was 4.36 g/kg bw/d for the males and 6.50 g/kg bw/d for the females. Roquette cites published gastrointestinal tolerance studies in humans that showed that 45 g/d was well-tolerated in male volunteers. Roquette also cites a published human study which examined mineral absorption and retention (magnesium, calcium, and zinc). There were no adverse effects on mineral absorption and retention in male volunteers who consumed from 20 g/d up to 100 g/d over 31 days. Roquette states that the two products are chemically similar to each other with the exception that the product tested has a lower level of mono- and disaccharide sugars and higher levels of fiber and therefore the cited studies are relevant for both products.”

As taken from FDA, 2013.

“BACKGROUND: Prebiotic fibers stimulate the growth and activity of the gut microbiota. Interleukin 10-deficient (IL-10(-/-)) mice develop a colitis that is influenced by the gut microbial composition. OBJECTIVE: The purpose of this study was to determine the effect of prebiotic fibers on the intestinal microbiota and immune function in IL-10(-/-) mice. METHODS: At 4 wk of age, male IL-10(-/-) mice (n = 8/group) were randomly assigned to 5 diets: unpurified diet with cellulose (4%; control), corn-derived hydroxypropylated new resistant starch (NRS) (2% NRS + 2% cellulose), soluble fiber dextrin from tapioca (SFD-t) (4%), soluble fiber dextrin from corn (SFD-c) (4%), or soluble corn fiber (4%) for 12 wk. Growth, small intestinal permeability, histologic injury, intestinal cytokine secretion, and microbiota composition by 16S ribosomal RNA pyrosequencing of stool were measured. ANOVA and principal component analysis were applied to assess the fibers' effects. RESULTS: There were no significant differences in mouse growth, intestinal weight, length, or gut permeability over the 12 wk feeding period. Mice fed dextrin-based diets secreted 47-88% less colonic IL-1 β , tumor necrosis factor α , and IL-23 (SFD-t diet) and IL-12 heterodimer p70, IL-6, and chemokine ligand 1 (CXCL1) (SFD-c diet) (P<0.05) than did the control group, whereas NRS-fed mice secreted 55-77% less IL-6 and CXCL1 (P<0.05). Both SFD-t- and SFD-c-fed mice had a 70-75% lower abundance of Lactobacillaceae than control mice. The SFD-t diet group had a lower enterocyte injury score (P<0.04) than did control mice, and this was associated with increased abundance of butyrate producers, including Incertae sedis XIV, Lachnospiraceae, and Ruminococcaceae (P<0.001). CONCLUSIONS: These results demonstrate that soluble prebiotic fibers selectively stimulate the growth of a distinctive gut microbiota in IL-10(-/-) mice. SFD-t induced the growth of butyrate-producing microbes and was effective in reducing proinflammatory cytokine secretion and enterocyte injury in this mouse model of colitis.” As taken from Valcheva R et al. 2015. J. Nutr. 145(9), 2060-6. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/?term=26180249>

5.3. Reproduction toxicity

Quantitative Risk Type - Not calculated

Quantitative Risk Value - Not calculated

Product Use – Not specified

Safety Evaluation Owner - COSMOS TTC (NON-CANCER)

POD Method - NOAEL

POD Value – 10000.0

POD Owner - COSMOS TTC (NON-CANCER)

Critical study - RAT (Reproductive/Developmental Toxicity) Oral - dietary exposure for 1 GEN

NOEL/LEL Owner - PAFA

Original NOEL - 10000.0 mg/kg bw/day

Original LEL – Not established

Critical Effects – NO EFFECTS

As taken from the COSMOS database available at <https://ng.cosmosdb.eu>

5.4. Mutagenicity

Test System	AMES SALMONELLA TYPHIMURIUM
Strain Indicator	TA98
Metabolic Activation	NONE
Method	STANDARD PLATE
Dose	40-5000 UG/PLATE (INDIGESTIBLE DEXTRIN)
Results	NEGATIVE
Reference	[WAKABAYASHI,S, SATOUCHI,M, UEDA,Y AND OHKUMA,K; ACUTE TOXICITY AND MUTAGENICITY STUDIES OF INDIGESTIBLE DEXTRIN, AND ITS EFFECT ON BOWEL MOVEMENT OF THE RAT; SHOKUHIN EISEIGAKU ZASSHI 33(6):557-562, 1992].
Test System	AMES SALMONELLA TYPHIMURIUM
Strain Indicator	TA98
Metabolic Activation	RAT, LIVER, S-9
Method	STANDARD PLATE

Dose	40-5000 UG/PLATE (INDIGESTIBLE DEXTRIN)
Results	NEGATIVE
Reference	[WAKABAYASHI,S, SATOUCHI,M, UEDA,Y AND OHKUMA,K; ACUTE TOXICITY AND MUTAGENICITY STUDIES OF INDIGESTIBLE DEXTRIN, AND ITS EFFECT ON BOWEL MOVEMENT OF THE RAT; SHOKUHIN EISEIGAKU ZASSHI 33(6):557-562, 1992].
Test System	AMES SALMONELLA TYPHIMURIUM
Strain Indicator	TA100
Metabolic Activation	NONE
Method	STANDARD PLATE
Dose	40-5000 UG/PLATE (INDIGESTIBLE DEXTRIN)
Results	NEGATIVE
Reference	[WAKABAYASHI,S, SATOUCHI,M, UEDA,Y AND OHKUMA,K; ACUTE TOXICITY AND MUTAGENICITY STUDIES OF INDIGESTIBLE DEXTRIN, AND ITS EFFECT ON BOWEL MOVEMENT OF THE RAT; SHOKUHIN EISEIGAKU ZASSHI 33(6):557-562, 1992].
Test System	AMES SALMONELLA TYPHIMURIUM
Strain Indicator	TA100
Metabolic Activation	RAT, LIVER, S-9
Method	STANDARD PLATE
Dose	40-5000 UG/PLATE (INDIGESTIBLE DEXTRIN)
Results	NEGATIVE

Reference	[WAKABAYASHI,S, SATOUCHI,M, UEDA,Y AND OHKUMA,K; ACUTE TOXICITY AND MUTAGENICITY STUDIES OF INDIGESTIBLE DEXTRIN, AND ITS EFFECT ON BOWEL MOVEMENT OF THE RAT; SHOKUHN EISEIGAKU ZASSHI 33(6):557-562, 1992].
Test System	AMES SALMONELLA TYPHIMURIUM
Strain Indicator	TA1535
Metabolic Activation	NONE
Method	STANDARD PLATE
Dose	40-5000 UG/PLATE (INDIGESTIBLE DEXTRIN)
Results	NEGATIVE
Reference	[WAKABAYASHI,S, SATOUCHI,M, UEDA,Y AND OHKUMA,K; ACUTE TOXICITY AND MUTAGENICITY STUDIES OF INDIGESTIBLE DEXTRIN, AND ITS EFFECT ON BOWEL MOVEMENT OF THE RAT; SHOKUHN EISEIGAKU ZASSHI 33(6):557-562, 1992].
Test System	AMES SALMONELLA TYPHIMURIUM
Strain Indicator	TA1535
Metabolic Activation	RAT, LIVER, S-9
Method	STANDARD PLATE
Dose	40-5000 UG/PLATE (INDIGESTIBLE DEXTRIN)
Results	NEGATIVE
Reference	[WAKABAYASHI,S, SATOUCHI,M, UEDA,Y AND OHKUMA,K; ACUTE TOXICITY AND MUTAGENICITY STUDIES OF INDIGESTIBLE DEXTRIN, AND ITS EFFECT ON BOWEL MOVEMENT OF THE RAT; SHOKUHN EISEIGAKU ZASSHI 33(6):557-562, 1992].

Test System	AMES SALMONELLA TYPHIMURIUM
Strain Indicator	TA1537
Metabolic Activation	NONE
Method	STANDARD PLATE
Dose	40-5000 UG/PLATE (INDIGESTIBLE DEXTRIN)
Results	NEGATIVE
Reference	[WAKABAYASHI,S, SATOUCHI,M, UEDA,Y AND OHKUMA,K; ACUTE TOXICITY AND MUTAGENICITY STUDIES OF INDIGESTIBLE DEXTRIN, AND ITS EFFECT ON BOWEL MOVEMENT OF THE RAT; SHOKUHIN EISEIGAKU ZASSHI 33(6):557-562, 1992].
Test System	AMES SALMONELLA TYPHIMURIUM
Strain Indicator	TA1537
Metabolic Activation	RAT, LIVER, S-9
Method	STANDARD PLATE
Dose	40-5000 UG/PLATE (INDIGESTIBLE DEXTRIN)
Results	NEGATIVE
Reference	[WAKABAYASHI,S, SATOUCHI,M, UEDA,Y AND OHKUMA,K; ACUTE TOXICITY AND MUTAGENICITY STUDIES OF INDIGESTIBLE DEXTRIN, AND ITS EFFECT ON BOWEL MOVEMENT OF THE RAT; SHOKUHIN EISEIGAKU ZASSHI 33(6):557-562, 1992].
Test System	E. COLI
Strain	WP2 UVRA

Indicator	
Metabolic Activation	NONE
Method	STANDARD PLATE
Dose	40-5000 UG/PLATE (INDIGESTIBLE DEXTRIN)
Results	NEGATIVE
Reference	[WAKABAYASHI,S, SATOUCHI,M, UEDA,Y AND OHKUMA,K; ACUTE TOXICITY AND MUTAGENICITY STUDIES OF INDIGESTIBLE DEXTRIN, AND ITS EFFECT ON BOWEL MOVEMENT OF THE RAT; SHOKUHIN EISEIGAKU ZASSHI 33(6):557-562, 1992].
Test System	E. COLI
Strain Indicator	WP2 UVRA
Metabolic Activation	RAT, LIVER, S-9
Method	STANDARD PLATE
Dose	40-5000 UG/PLATE (INDIGESTIBLE DEXTRIN)
Results	NEGATIVE
Reference	[WAKABAYASHI,S, SATOUCHI,M, UEDA,Y AND OHKUMA,K; ACUTE TOXICITY AND MUTAGENICITY STUDIES OF INDIGESTIBLE DEXTRIN, AND ITS EFFECT ON BOWEL MOVEMENT OF THE RAT; SHOKUHIN EISEIGAKU ZASSHI 33(6):557-562, 1992].

As taken from CCRIS, 1995.

“Roquette notes that the toxicological and human studies on enzyme-modified dextrin were performed using the product containing the higher fiber level. Roquette cites a published study that reported the results of an Ames assay...(and a)....mouse lymphoma assay....The results from Ames and mouse lymphoma assays showed that enzyme-modified dextrin has no mutagenic activity.”

As taken from FDA, 2013.

5.5. Cytotoxicity

"The uptake of nanoparticles by cells of the mononuclear phagocytic system limits its use as colloidal drug carriers, reducing the blood circulation time and the ability to reach biological targets. In this work, the interaction between dextrin nanoparticles--recently developed in our laboratory--and murine bone marrow-derived macrophages was evaluated. Cytotoxicity were studied, using the MTT assay The results show that the nanoparticles are not cytotoxic" (Goncalves et al. 2010b).

5.6. Carcinogenicity

The influence of simple sugars and starch given during pre- or post-initiation on aberrant crypt foci in rat colon (Abstract). The aim of one study was to investigate the enhancing effect of dietary sugar on the development of aberrant crypt foci (ACF) in male F344 rats initiated with azoxymethane (AOM). The potential role of sugar as either a co-initiator or a promoter was investigated by giving diets high in sucrose and dextrin (61%) during either the pre-initiation, the initiation, and/or the post-initiation stage of the ACF development. The colonic cell proliferation, activity of colonic phase II enzymes, and a biomarker of lipid peroxidation were additionally examined in order to obtain information on the specific mechanisms involved in the suggested effect of sucrose and dextrin on ACF development. The number of large sized and the total number of ACF were significantly increased by feeding sucrose and dextrin in the post-initiation period. No positive association between colonic cell proliferation and ACF was seen. The level of oxidative stress in the cytosol from the proximal colon and colonic glutathione transferase and quinone reductase was not affected by the sugar treatments. The overall results from this study show that sucrose and dextrin enhance the number of preneoplastic lesions in AOM-initiated rats, and act primarily as promoters in the development of ACF. Poulsen M, Mølck AM, Thorup I, Breinholt V, Meyer O. Cancer Lett. 2001, Jun 26; 167(2):135-43. As taken from PubMed, 2010 available at <http://www.ncbi.nlm.nih.gov/pubmed/11369133>

5.7. Irritation/immunotoxicity

May cause irritation (Haz-Map, 2020).

"Dextrin: Rinse-off facial product containing 42.6919 % dextrin (1% aqueous; effective concentration $\approx$ 0.4%). 54 subjects (46 females, 8 males). HRIPT. During induction, product (0.1-0.15 g on occlusive patch) applied for 24 h to the back.. Dose/concentration per cm² not stated. Challenge patch applied to new test site and reactions scored at 24 h and 72 h post-application. Transient, barely perceptible erythema, in 1 subject, during induction. No reactions observed during challenge phase. No clinically significant skin irritation or evidence of allergic contact dermatitis".

As taken from CIR, 2015. Available at <http://www.cir-safety.org/sites/default/files/plpogu092015final.pdf>

"BACKGROUND: Prebiotic fibers stimulate the growth and activity of the gut microbiota. Interleukin 10-deficient (IL-10(-/-)) mice develop a colitis that is influenced by the gut microbial composition. OBJECTIVE: The purpose of this study was to determine the effect of prebiotic fibers on the intestinal microbiota and immune function in IL-10(-/-) mice. METHODS: At 4 wk of age, male IL-10(-/-) mice (n = 8/group) were randomly assigned to 5 diets: unpurified diet with cellulose (4%; control), corn-derived hydroxypropylated new resistant starch (NRS) (2% NRS + 2% cellulose), soluble fiber dextrin from tapioca (SFD-t) (4%), soluble fiber dextrin from corn (SFD-c) (4%), or soluble corn fiber (4%) for 12 wk. Growth, small intestinal permeability, histologic injury, intestinal cytokine secretion, and microbiota composition by 16S ribosomal RNA pyrosequencing of stool were measured. ANOVA and principal component analysis were applied to assess the fibers' effects. RESULTS: There were no significant differences in mouse growth, intestinal weight, length, or gut permeability over the 12 wk feeding period. Mice fed dextrin-based diets secreted 47-88% less colonic IL-1 β , tumor necrosis factor α , and IL-23 (SFD-t diet) and IL-12 heterodimer p70, IL-6,

and chemokine ligand 1 (CXCL1) (SFD-c diet) ($P < 0.05$) than did the control group, whereas NRS-fed mice secreted 55-77% less IL-6 and CXCL1 ($P < 0.05$). Both SFD-t- and SFD-c-fed mice had a 70-75% lower abundance of Lactobacillaceae than control mice. The SFD-t diet group had a lower enterocyte injury score ($P < 0.04$) than did control mice, and this was associated with increased abundance of butyrate producers, including Incertae sedis XIV, Lachnospiraceae, and Ruminococcaceae ($P < 0.001$). CONCLUSIONS: These results demonstrate that soluble prebiotic fibers selectively stimulate the growth of a distinctive gut microbiota in IL-10(-/-) mice. SFD-t induced the growth of butyrate-producing microbes and was effective in reducing proinflammatory cytokine secretion and enterocyte injury in this mouse model of colitis." As taken from Valcheva R et al. 2015. J. Nutr. 145(9), 2060-6. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/?term=26180249>

"OBJECTIVE: The aim of this trial was to determine the efficacy of a resistant dextrin on immune-mediated inflammation and hypothalamic-pituitary-adrenal axis in women with type 2 diabetes mellitus (T2DM). METHODS: Females ($n = 55$) with T2DM were randomly allocated into intervention group ($n = 30$) and control group ($n = 25$), in which they received 10 g/d of Nutriose®06 (a resistant dextrin) or maltodextrin for 8 weeks, respectively. Fasting blood samples were taken to measure immune system related parameters like white blood cell count, CD4, CD8, interferon- γ (IFN γ), interleukins (IL12, IL4, IL10), cortisol, tryptophan (TRP), ACTH (Adrenocorticotrophic hormone), Kynurenine (KYN) and plasma lipopolysaccharide (LPS) at the beginning and end of trial. Mental health was assessed using general health questionnaire (GHQ) and depression, anxiety and stress scale (DASS). RESULTS: Resistant dextrin caused a significant decrease in levels of cortisol, KYN, KYN/TRP ratio, IFN γ , IL12, IFN γ /IL10 ratio, LPS, and a significant increase in the monocyte, GHQ, DASS, CD8, IL10, IL4 in the intervention group as compared with baseline. A significant decrease in the level of LPS (-6.20 EU/mL, -17.8%), IFN γ (-0.6 pg/ml, -26.8%), cortisol (-2.6 μ g/dl, -20.9%), IFN γ /IL10 ratio (0.01, 10%), GHQ (-5.1, -12.5%), DASS (-10.4, -38.4%), KYN/TRP ratio (6.8, 29.1%), and a significant increase in levels of CD8 (6.4%, 6.1%) and IL10 (2.6 pg/ml, 21.6%) in the intervention group as compared with the control group ($P < 0.05$). No significant changes were observed in white blood cell count, CD4, CD4/CD8 ratio, ACTH, KYN, TRP, IL4 and IL12 in the intervention group as compared with the control group ($P > 0.05$). CONCLUSION: Supplementation of Nutriose®06 may have beneficial effects on mental health and the immune system response in women with T2DM." As taken from Farhangi MA et al. 2018. Clin. Nutr. 37(4), 1216-1223. PubMed, 2019 available at: <https://www.ncbi.nlm.nih.gov/pubmed/28669666>

5.8. All other relevant types of toxicity

"Effects of the in ovo injection of commercial diluent supplemented with dextrin or with dextrin in combination with various other carbohydrates on the somatic characteristics and liver nutrient profiles of Ross $\times$ Ross 708 broiler embryos and chicks were investigated. Results include information concerning the gluconeogenic energy status of the liver before and after hatch. Eggs containing live embryos were injected in the amnion on d 18 of incubation using an automated multiple-egg injector for the delivery of the following carbohydrates dissolved in 0.4 mL of commercial diluent: 1) 6.25% glucose and 18.75% dextrin; 2) 6.25% sucrose and 18.75% dextrin; 3) 6.25% maltose and 18.75% dextrin; and 4) 25% dextrin. Also, a noninjected control and a 0.4-mL diluent-injected control were included. Body weight relative to set egg weight on d 19 of incubation (E19) was increased by the injection of all carbohydrate solutions, and on the day of hatch was increased by the injection of diluent, sucrose and dextrin, and maltose and dextrin solutions. Hatchability of the fertilized eggs, residual yolk sac weight, and liver weight were not affected by any injection treatment; however, as compared with the 0.4 mL diluent-injected group, all of the supplementary carbohydrates, except for the glucose and dextrin combination group, increased liver glycogen and glucose concentrations on E19. Furthermore, all carbohydrates, except for the 25% dextrin treatment, decreased liver fat concentration on E19. From E19 to the day of hatch, liver glycogen concentrations dropped dramatically from an average of 3.2 to 0.6%.

Despite treatment differences observed on E19 for liver glycogen, glucose, and fat concentrations, these differences were lost by the day of hatch. Nevertheless, liver glycogen and glucose concentrations were positively correlated on the day of hatch. In conclusion, the in ovo injection of various supplemental carbohydrates dissolved in 0.4 mL of commercial diluent altered the liver nutrient profile of Ross × Ross 708 broiler embryos before hatch. However, the subsequent pattern of energy utilization during the hatching process modified these effects” (Zhai et al. 2011a).

“The effects of the in ovo injection of different carbohydrate solutions on the internal egg temperature (IT), hatchability, and time of hatch of embryonated Ross × Ross 708 broiler hatching eggs were determined. In addition, the BW, liver weight, yolk sac weight (YSW), and yolk-free BW (YFBW) of the embryos on d 19.5 of incubation and of the chicks on day of hatch were determined. Eggs containing live embryos were injected in the amnion on d 18.5 of incubation using an automated multiple-egg injector. Solution injections delivered 1.2 mL of physiological saline (0.85%) alone or with a supplemental carbohydrate. The following supplemental carbohydrates were separately dissolved in saline at a concentration of 0.3 g/mL: glucose, fructose, sucrose, maltose, and dextrin. Temperature transponders were implanted in the air cells of embryonated and nonembryonated eggs after in ovo injection for the detection of IT at 6, 14, and 22 h after injection. The IT of embryonated eggs was significantly greater than that of nonembryonated eggs at all 3 times after the treatment period. Eggs that were injected with saline with or without supplemental carbohydrates experienced a reduction in IT when compared with control eggs whose shells were perforated without solution delivery, and the decrease in IT was associated with a delay in hatch time. Liver weight was negatively related to YSW and positively related to YFBW, and YSW was negatively related to YFBW. Although the saline and carbohydrate solution injections increased chick BW compared with noninjected controls, chick YFBW was decreased in the maltose- and sucrose-injected groups. In conclusion, the injection of 1.2 mL of saline with or without supplemental carbohydrates lowered embryonic metabolism, as reflected by a lower IT and a delay in time of hatch. However, effects of the different carbohydrate solutions on yolk absorption and tissue deposition in yolk-free embryos varied. These results suggest that lower volumes for solutions containing maltose, sucrose, or fructose should be considered for in ovo injection” (Zhai et al. 2011b).

“The effects of in ovo injection of different carbohydrate solutions on hatchability of fertilized eggs (HF), rate of hatch, BW, body moisture, yolk sac weight, and yolk sac moisture of Ross × 708 broiler chicks, hatched from eggs laid by a 34-wk-old breeder flock, were investigated. Eggs containing live embryos were injected, using an automated multiple-egg injector, in the amnion on d 18.5 of incubation with 0.1, 0.4, 0.7, or 1.0 mL of commercial diluent or a carbohydrate dissolved in diluent. The commercial diluent containing 0.25 g/mL of one of the following carbohydrates was injected into eggs: glucose, fructose, sucrose, maltose, or dextrin. The results showed that no carbohydrate type or solution volume affected rate of hatch. Absolute and proportional BW on day of hatch were positively related to injection volume ($P < 0.001$). However, HF was negatively related to injection volume ($P < 0.001$). To realize an HF of 90%, the injection volume could not exceed 0.4 mL for fructose or sucrose and could not exceed 0.7 mL for glucose, maltose, or dextrin. Yolk-free BW was negatively related to injection volume of fructose and sucrose ($P < 0.004$), but was not related to injection volume of diluent, glucose, maltose, and dextrin. Conversely, absolute and proportional yolk sac weights were positively related to injection volume of fructose, sucrose, and dextrin ($P < 0.01$), but were also not significantly related to injection volume of diluent, glucose, and maltose. Yolk sac moisture was positively related to injection volume for all injectables, including the diluent ($P < 0.03$). However, body moisture and yolk-free body moisture were not related to injection type or volume. In conclusion, the use of carbohydrates added to a commercial diluent for the in ovo injection of broiler hatching eggs requires the use of appropriate volumes to promote growth and nutrient utilization without adversely affecting HF” (Zhai et al. 2011c).

“The health benefits of dietary fiber have long been appreciated. Higher intakes of dietary fiber are linked to less cardiovascular disease and fiber plays a role in gut health, with many effective laxatives actually isolated fiber sources. Higher intakes of fiber are linked to lower body weights.

Only polysaccharides were included in dietary fiber originally, but more recent definitions have included oligosaccharides as dietary fiber, not based on their chemical measurement as dietary fiber by the accepted total dietary fiber (TDF) method, but on their physiological effects. Inulin, fructo-oligosaccharides, and other oligosaccharides are included as fiber in food labels in the US. Additionally, oligosaccharides are the best known "prebiotics", "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health." To date, all known and suspected prebiotics are carbohydrate compounds, primarily oligosaccharides, known to resist digestion in the human small intestine and reach the colon where they are fermented by the gut microflora. Studies have provided evidence that inulin and oligofructose (OF), lactulose, and resistant starch (RS) meet all aspects of the definition, including the stimulation of *Bifidobacterium*, a beneficial bacterial genus. Other isolated carbohydrates and carbohydrate-containing foods, including galactooligosaccharides (GOS), transgalactooligosaccharides (TOS), polydextrose, wheat dextrin, acacia gum, psyllium, banana, whole grain wheat, and whole grain corn also have prebiotic effects." As taken from Slavin J. 2013. *Nutrients* 5(4), 1417-35. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23609775>

“BACKGROUND: Dessert compositions may conform to diabetic diet when it contains low sugar or artificial sweetener to replace sugar. However, it is still questionable whether glycemic control in type 2 diabetes patients is improved by the use of diet-conforming dessert compositions. **OBJECTIVE:** To compare, in type 2 diabetes patients, the glycemic, insulin, and C-peptide responses to seven modified dessert compositions for diabetics (D-dessert) with the response to seven similar desserts of non-modified composition, used as control desserts (C-dessert). **METHODS:** Seventy type 2 diabetes patients were allocated to seven groups of ten. On three occasions, each patient received either the meal which consisted of bread and cheese, or the meal and D-dessert, or the meal and the respective C-dessert. Differences in postprandial glucose, insulin, and C-peptide were evaluated using analysis of repeated measures at 0, 30, 60, 90, and 120 min after consumption. **RESULTS:** D-cake and D-pastry cream resulted in lower glucose levels (8.81 ± 0.32 mmol/l and 8.67 ± 0.36 mmol/l, respectively) and D-strawberry jelly in lower insulin levels (16.46 ± 2.66 μ U/ml) than the respective C-desserts (9.99 ± 0.32 mmol/l for C-cake, 9.28 ± 0.36 mmol/l for C-pastry cream, and 27.42 ± 2.66 μ U/ml for C-strawberry jelly) ($P < 0.05$). Compared with the meal, D-cake did not increase glucose or insulin levels ($P < 0.05$), while C-cake did ($P < 0.05$). D-pastry cream increased glucose to a lesser extent than C-pastry cream ($P < 0.05$). Similar effects were reported for D-milk dessert, D-millefeuille, and D-chocolate on glucose, insulin, and C-peptide at specific timepoints. D-crème caramel showed no effect. **CONCLUSIONS:** Some desserts formulated with sugar substitutes and soluble fiber may have a favorable effect on postprandial levels of glucose, insulin, and C-peptide in type 2 diabetic patients.” As taken from Argyri K et al. 2013. *Rev. Diabet. Stud.* 10(1), 39-48. PubMed, 2015 available at: <http://www.ncbi.nlm.nih.gov/pubmed/24172697>

“In the present study, enzyme-resistant dextrin, prepared by heating of potato starch in the presence of hydrochloric (0.1% dsb) and citric (0.1% dsb) acid at 130°C for 3 h (CA-dextrin), was tested as a source of carbon for probiotic lactobacilli and bifidobacteria cultured with intestinal bacteria isolated from feces of three healthy 70-year old volunteers. The dynamics of growth of bacterial monocultures in broth containing citric acid (CA)-modified dextrin were estimated. It was also investigated whether lactobacilli and bifidobacteria cultured with intestinal bacteria in the presence of resistant dextrin would be able to dominate the intestinal isolates. Prebiotic fermentation of resistant dextrin was analyzed using prebiotic index (PI). In co-cultures of intestinal and probiotic bacteria, the environment was found to be dominated by the probiotic strains of *Bifidobacterium* and *Lactobacillus*, which is a beneficial effect.” As taken from Slizewska K. 2013. *Acta Biochim. Pol.* 60(4), 671-5. PubMed, 2015 available at: <http://www.ncbi.nlm.nih.gov/pubmed/24432315>

“BACKGROUND: Nutritional therapy is an important component of diabetes management. There is data to suggest that fiber content of foods may affect glycemic response. **MATERIALS AND**

METHODS: 10 children, diagnosed with type 1 diabetes, participated. In the first phase of the study, children followed their usual meal plan. In the second phase, subjects followed the same meal plan except that fiber was added to the diet using a powder supplement (wheat dextrin). Data was collected using a continuous glucose monitoring device. The blood glucose excursion level following each meal was compared between the two phases of the study by fitting a repeated measures regression model. The incidence of hypoglycemia was also compared by fitting a logistic regression model. **RESULTS:** There was no difference in the mean blood glucose excursion after meals or the incidence of hypoglycemia between the two phases. There was a strong negative correlation between the amount of fiber supplemented and the mean maximum post-prandial blood sugar after the lunch and breakfast meals (Spearman rank correlation coefficient = -0.86 lunch and -0.76 breakfast). **CONCLUSION:** Our study did not show an overall decrease in glucose excursion or incidence of hypoglycemia with fiber supplementation. We did find a strong negative correlation between the amount of fiber added during the supplemental phase and the mean maximum post-prandial blood sugar after the lunch and breakfast meals. We speculate that different types of fiber may have different effects on blood glucose with wheat dextrin having a greater dampening effect.” As taken from Nader N et al. 2014. *Int. J. Pediatr. Endocrinol.* 2014(1), 13. PubMed, 2015 available at: <http://www.ncbi.nlm.nih.gov/pubmed/25024710>

“**BACKGROUND:** Prebiotic fibers stimulate the growth and activity of the gut microbiota. Interleukin 10-deficient (IL-10(-/-)) mice develop a colitis that is influenced by the gut microbial composition. **OBJECTIVE:** The purpose of this study was to determine the effect of prebiotic fibers on the intestinal microbiota and immune function in IL-10(-/-) mice. **METHODS:** At 4 wk of age, male IL-10(-/-) mice (n = 8/group) were randomly assigned to 5 diets: unpurified diet with cellulose (4%; control), corn-derived hydroxypropylated new resistant starch (NRS) (2% NRS + 2% cellulose), soluble fiber dextrin from tapioca (SFD-t) (4%), soluble fiber dextrin from corn (SFD-c) (4%), or soluble corn fiber (4%) for 12 wk. Growth, small intestinal permeability, histologic injury, intestinal cytokine secretion, and microbiota composition by 16S ribosomal RNA pyrosequencing of stool were measured. ANOVA and principal component analysis were applied to assess the fibers' effects. **RESULTS:** There were no significant differences in mouse growth, intestinal weight, length, or gut permeability over the 12 wk feeding period. Mice fed dextrin-based diets secreted 47-88% less colonic IL-1 β , tumor necrosis factor α , and IL-23 (SFD-t diet) and IL-12 heterodimer p70, IL-6, and chemokine ligand 1 (CXCL1) (SFD-c diet) (P<0.05) than did the control group, whereas NRS-fed mice secreted 55-77% less IL-6 and CXCL1 (P<0.05). Both SFD-t- and SFD-c-fed mice had a 70-75% lower abundance of Lactobacillaceae than control mice. The SFD-t diet group had a lower enterocyte injury score (P<0.04) than did control mice, and this was associated with increased abundance of butyrate producers, including Incertae sedis XIV, Lachnospiraceae, and Ruminococcaceae (P<0.001). **CONCLUSIONS:** These results demonstrate that soluble prebiotic fibers selectively stimulate the growth of a distinctive gut microbiota in IL-10(-/-) mice. SFD-t induced the growth of butyrate-producing microbes and was effective in reducing proinflammatory cytokine secretion and enterocyte injury in this mouse model of colitis.” As taken from Valcheva R et al. 2015. *J. Nutr.* 145(9), 2060-6. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/?term=26180249>

“**INTRODUCTION:** Polycystic ovary syndrome (PCOS) is one of the most common abnormalities in women of reproductive age that can lead to a variety of metabolic and reproductive disorders. Studies reveal that a healthy diet is the most effective way for treating the risk factors associated with metabolic disorders and place greater emphasis on the consumption of prebiotic foods. The present study aims to determine the effect of resistant Dextrin on metabolic parameters, including lipid profile, fasting blood glucose (FBS) and high sensitivity C-reactive protein (hsCRP), and androgen levels, including serum levels of dehydroepiandrosterone sulfate (DHEA-S) and free testosterone, as the primary outcomes, and manifestations of PCOS including menstrual cycle irregularity and hirsutism, as the secondary outcomes.

METHODS: This randomized, controlled, triple-blind, clinical trial was conducted on 62 women aged 18-45 in Tabriz, Iran, in 2016-2017. The participants were divided into a prebiotic group and a placebo group using block randomization. The prebiotic group consumed 20 g of resistant dextrin dissolved in a glass of water and the placebo group 20 g of maltodextrin also dissolved in a glass of water on a daily basis for 3 months. To measure the serum lipid profile, FBS, hsCRP, DHEA-S and free testosterone before and 3 months after the intervention, 5-ml blood samples were collected from the participants and analyzed using the ELISA method. The Ferriman-Gallwey scale for assessing hirsutism and a checklist for assessing menstrual cycle characteristics were completed before and 3 months after the intervention. A general linear model was used to analyze the data. **RESULTS:** No statistically significant differences were observed between the two groups in terms of sociodemographic characteristics and baseline values. 3 months after the intervention, based on the ANCOVA and after adjusting for the baseline values, the mean serum levels of LDL-C (adjusted mean difference = - 29.79; 95% CI = - 43.37 to - 16.21; $P < 0.001$), triglyceride (AMD = - 38.50; 95% CI = - 59.73 to - 17.28; $P = 0.001$), total cholesterol (AMD = - 29.98; 95% CI = - 40.14 to - 19.82; $P < 0.001$), FBS (AMD = - 11.24; 95% CI = - 15.43 to - 7.06; $P < 0.001$), hsCRP (AMD = - 1.75; 95% CI = - 2.92 to - 0.57; $P = 0.004$), DHEA-S (AMD = - 0.7; 95% CI = - 1.34 to - 0.13; $P = 0.017$) and free testosterone (AMD = - 0.32; 95% CI = - 0.56 to - 0.08; $P = 0.010$) revealed a statistically significant decrease in the intervention group compared to the placebo group, while the mean serum HDL-C showed a statistically significant increase in this group compared to the placebo group (AMD = 5.82; 95% CI = 2.27-9.37; $P = 0.002$). 3 months after the intervention, there was a significant difference between the two groups in terms of menstrual cycle intervals and hirsutism ($P < 0.001$). **CONCLUSION:** Resistant dextrin consumption can regulate metabolic parameters and androgen levels and manifestations including hirsutism and menstrual cycle irregularity in women with PCOS." As taken from Gholizadeh Shamasbi S et al. 2019. Eur. J. Nutr. 58(2), 629-640.. PubMed, 2019 available at: <https://www.ncbi.nlm.nih.gov/pubmed/29480399>

"Obesity is a leading public health problem throughout the world. The development of foods that increase satiety and reduce food may aid weight management. This study determined the effect of consuming soluble fiber dextrin (SFD) on appetite, appetitive hormones, breath hydrogen and food intake in adults. Forty-three participants completed this study. For each treatment, 50% of the SFD was provided in liquid form as part of breakfast and 50% in solid form as a morning snack. Appetite questionnaires, blood and breath samples were collected immediately before breakfast and at regular intervals during the test session. The participants consumed an ad libitum lunch meal, afternoon snack and dinner meal, and the amount eaten was recorded. Following dinner, participants left the laboratory but were required to keep a diet diary for the remainder of the day. Breath hydrogen concentration was significantly higher following the consumption of SFD compared to control ($p < 0.05$). There was no observed overall treatment effect of consuming SFD on GLP-1 (Glucagon-Like-Peptide-1), ghrelin, CCK-8 (Cholecystokinin) or PYY3-36 (Peptide YY) ($p > 0.05$). Moreover, consuming foods containing SFD had no effect on subjective appetite or food intake ($p > 0.05$). Consuming foods containing SFD increased breath hydrogen but did not influence food intake, appetite or appetitive hormones. However, the limitations of this study may have individually or collectively masked an effect of SFD on food intake and appetite." As taken from Emilien CH et al. 2020. Nutrients 12(11), 3341. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/33143121/>

"Although glucose is the best-known nutrient to stimulate glucagon-like peptide-1 (GLP-1) secretion, dietary peptides also potently stimulate GLP-1 secretion. Certain peptide fragments derived from dietary proteins possess dipeptidyl peptidase-4 (DPP-4) inhibitory activity in vitro. Hence, we hypothesised that dietary peptides protect GLP-1 from degradation through attenuating DPP-4 activity in vivo. Here, we compared GLP-1 responses with dietary proteins, a carbohydrate and a lipid (Intralipos) in rats having or not having plasma DPP-4 activity. Plasma GLP-1 concentrations clearly increased by oral administration of whey protein (2-4 g/kg), but not by that of dextrin (2-4 g/kg), in control rats (untreated Sprague-Dawley rats and F344/Jcl rats), having DPP-4 activity. In contrast, dextrin administration increased the plasma GLP-1 concentrations as the whey

protein administration did, in rats having reduced or no DPP-4 activity (a DPP-4 inhibitor, sitagliptin-treated Sprague-Dawley rats or DPP-4-deficient F344/DuCrI/CrIj rats). DPP-4 inhibition by sitagliptin treatment also enhanced GLP-1 response to Intralipos, and casein, but the treatment did not further enhance GLP-1 response to whey protein. Intestinal GLP-1 content and gastric emptying rate were not associated with differences in GLP-1 responses to test nutrients. The luminal contents from rats administered whey protein decreased DPP-4 activity in vitro. These results suggest that GLP-1 released by dextrin, Intralipos and casein was immediately degraded by DPP-4, while GLP-1 released by whey protein was less degraded. Our study provides novel in vivo evidence supporting the hypothesis that dietary peptides not only stimulate GLP-1 secretion but also inhibit DPP-4 activity to potentiate GLP-1 response.” As taken from Shimizu Y et al. 2021. Br. J. Nutr. 125(4), 398-407. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/32713353/>

6. Functional effects on

6.1. Broncho/pulmonary system

No data available to us at this time.

6.2. Cardiovascular system

“Purpose: Resistant dextrin (RD) supplementation has been shown to alter satiety, glycaemia, and body weight, in overweight Chinese men; however, there are limited data on its effects in other demographic groups. Here, we investigated the effects of RD on satiety in healthy adults living in the United Kingdom. Methods: 20 normal weight and 16 overweight adults completed this randomised controlled cross-over study. Either RD (14 g/day NUTRIOSE® FB06) or maltodextrin control was consumed in mid-morning and mid-afternoon preload beverages over a 28-day treatment period with crossover after a 28-day washout. During 10-h study visits (on days 1, 14, and 28 of each treatment period), satietogenic, glycaemic and anorectic hormonal responses to provided meals were assessed. Results: Chronic supplementation with RD was associated with higher fasted satiety scores at day 14 ($P = 0.006$) and day 28 ($P = 0.040$), compared to control. RD also increased satiety after the mid-morning intervention drink, but it was associated with a reduction in post-meal satiety following both the lunch and evening meals ($P < 0.01$). The glycaemic response to the mid-morning intervention drink (0-30 min) was attenuated following RD supplementation ($P < 0.01$). Whilst not a primary endpoint we also observed lower systolic blood pressure at day 14 ($P = 0.035$) and 28 ($P = 0.030$), compared to day 1, following RD supplementation in the normal weight group. Energy intake and anthropometrics were unaffected. Conclusions: RD supplementation modified satiety and glycaemic responses in this cohort, further studies are required to determine longer-term effects on body weight control and metabolic markers.” As taken from Hobden MR et al. 2021. Eur. J. Nutr. Epub ahead of print. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/34170392/>

6.3. Nervous system

“OBJECTIVE: The aim of this trial was to determine the efficacy of a resistant dextrin on immune-mediated inflammation and hypothalamic-pituitary-adrenal axis in women with type 2 diabetes mellitus (T2DM). METHODS: Females ($n = 55$) with T2DM were randomly allocated into intervention group ($n = 30$) and control group ($n = 25$), in which they received 10 g/d of Nutriose®06 (a resistant dextrin) or maltodextrin for 8 weeks, respectively. Fasting blood samples were taken to measure immune system related parameters like white blood cell count, CD4, CD8, interferon- γ (IFN γ), interleukins (IL12, IL4, IL10), cortisol, tryptophan (TRP), ACTH (Adrenocorticotrophic hormone), Kynurenine (KYN) and plasma lipopolysaccharide (LPS) at the beginning and end of trial. Mental health was assessed using general health questionnaire (GHQ) and depression, anxiety and stress scale (DASS). RESULTS: Resistant dextrin caused a significant decrease in

levels of cortisol, KYN, KYN/TRP ratio, IFN γ , IL12, IFN γ /IL10 ratio, LPS, and a significant increase in the monocyte, GHQ, DASS, CD8, IL10, IL4 in the intervention group as compared with baseline. A significant decrease in the level of LPS (-6.20 EU/mL, -17.8%), IFN γ (-0.6 pg/ml, -26.8%), cortisol (-2.6 μ g/dl, -20.9%), IFN γ /IL10 ratio (0.01, 10%), GHQ (-5.1, -12.5%), DASS (-10.4, -38.4%), KYN/TRP ratio (6.8, 29.1%), and a significant increase in levels of CD8 (6.4%, 6.1%) and IL10 (2.6 pg/ml, 21.6%) in the intervention group as compared with the control group ($P < 0.05$). No significant changes were observed in white blood cell count, CD4, CD4/CD8 ratio, ACTH, KYN, TRP, IL4 and IL12 in the intervention group as compared with the control group ($P > 0.05$). CONCLUSION: Supplementation of Nutriose®06 may have beneficial effects on mental health and the immune system response in women with T2DM." As taken from Farhangi MA et al. 2018. Clin. Nutr. 37(4), 1216-1223. PubMed, 2019 available at: <https://www.ncbi.nlm.nih.gov/pubmed/28669666>

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

In a study on the effect of type of dietary carbohydrate on B-vitamin synthesis in the digestive tracts of rats, groups of 17 to 44 male and female rats (strain unspecified), 21 days of age were placed on test diets containing 18% protein (casein), 71% carbohydrate, and 3% butterfat, cod-liver oil and salt mixture. The carbohydrate sources used included corn starch, corn dextrin, glucose, lactose and sucrose. Animals on all diets, without supplemental B-vitamins but with access to their faeces, showed low or declining growth rates after 2 weeks except for the group fed the dextrin diet. Growth rates in all groups were increased after receiving faeces of the dextrin-fed group. Rats receiving the dextrin diet had enlarged caeca at the conclusion of the study. Caecotomized rats with access to their faeces lost weight when fed a dextrin diet; supplementation with baker's yeast resulted in weight gain. It was concluded that the peculiar property of corn dextrin was not due to retained B-vitamins, but rather to the formation of these vitamins in the lower part of the digestive tract of the rat as a result of incomplete digestion of this particular carbohydrate (Guerrant et al., 1935). [i.e Rats (strain unspecified), 21 days of age, were placed on test diets containing 18% protein (casein), 71% dextrin, and 3% butterfat, cod-liver oil and salt mixture. There was shown to be incomplete digestion of the dextrin (Guerrant et al., 1935).]

INCHEM (1982)

"OBJECTIVES: The prebiotic potential of NUTRIOSE®--a sugar-free, digestion-resistant dextrin--was evaluated in two randomized, placebo-controlled trials that included 48 and 40 healthy volunteers, respectively. METHODS: In study 1, the effect on colonic bacteria of NUTRIOSE® 10, 15 or 20 g/day administered for 14 days was examined; in study 2, gut microbial changes in response to NUTRIOSE® 8 g/day for 14 days were monitored using real-time polymerase chain reaction analysis. RESULTS: NUTRIOSE® increased proliferation of Bacteroides and inhibited Clostridium perfringens in both studies, increased β -glucosidase activity (at 10 and 15 g/day) and decreased colonic pH (at 20 g/day). The increase in short-chain fatty acid production with NUTRIOSE® consumption was not statistically significant. There were no indications of gastrointestinal intolerance at any dose. CONCLUSIONS: According to commonly accepted definitions, NUTRIOSE® is a prebiotic soluble fibre that provides a beneficial effect on colonic ecology while preserving digestive comfort". As taken from Lefranc-Millot C et al. (2012). J. Int. Med. Res. 40, 211-224. PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/22429361>

"Wheat dextrin soluble fibre may have metabolic and health benefits, potentially acting via mechanisms governed by the selective modulation of the human gut microbiota. Our aim was to examine the impact of wheat dextrin on the composition and metabolic activity of the gut microbiota. We used a validated in vitro three-stage continuous culture human colonic model (gut model) system comprised of vessels simulating anatomical regions of the human colon. To mimic human ingestion, 7 g of wheat dextrin (NUTRIOSE(®) FB06) was administered to three gut models, twice daily at 10.00 and 15.00, for a total of 18 days. Samples were collected and analysed for

microbial composition and organic acid concentrations by 16S rRNA-based fluorescence in situ hybridisation and gas chromatography approaches, respectively. Wheat dextrin mediated a significant increase in total bacteria in vessels simulating the transverse and distal colon, and a significant increase in key butyrate-producing bacteria *Clostridium* cluster XIVa and *Roseburia* genus in all vessels of the gut model. The production of principal short-chain fatty acids, acetate, propionate and butyrate, which have been purported to have protective, trophic and metabolic host benefits, were increased. Specifically, wheat dextrin fermentation had a significant butyrogenic effect in all vessels of the gut model and significantly increased production of acetate (vessels 2 and 3) and propionate (vessel 3), simulating the transverse and distal regions of the human colon, respectively. In conclusion, wheat dextrin NUTRIOSE(®) FB06 is selectively fermented in vitro by *Clostridium* cluster XIVa and *Roseburia* genus and beneficially alters the metabolic profile of the human gut microbiota." As taken from Hobden MR et al. 2013. PLoS One 8(10), e77128. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24204753>

"The purpose of this study was to examine the inhibitory effects of resistant starch on postprandial glycaemic response in obese dogs. The changes in blood glucose concentrations and glycaemic index (GI) were chronologically determined after the administration of resistant and normal starches by nasal feeding. Resistant starch contained indigestible dextrin (IDD) and β -cyclic dextrin (β -CD). Soluble starch (SS) served as a control starch. Glucose concentrations reached their maximum 15 min after the administration of SS solutions, and decreased gradually during the experimental period. In contrast, after the administration of IDD solutions, increased glucose concentrations rapidly decreased to the initial values. After the administration of β -CD solutions, glucose concentrations remained unchanged during this study. GI levels remained constant in the following order: β -CD <IDD <SS. GI levels of dogs receiving IDD and β -CD solutions were significantly lower as compared with those animals receiving SS solutions. In this study, nasal tube feeding was an effective method for evaluating glycaemic responses to various starches accurately. The present data revealed that resistant starches were useful materials in controlling nutritionally glucose concentrations in obese dogs. These results raise the possibility that resistant starches are valuable for dietetic treatment of diabetes and obesity in dogs." As taken from Kimura T. 2013. Arch. Anim. Nutr. 67(6), 503-9. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24228912>

"The objective of this study was to evaluate health outcomes resulting from dietary supplementation of novel, low-digestible carbohydrates in the cecum and colon of Sprague-Dawley rats randomly assigned to one of four treatment groups for 21 days: 5% cellulose (Control), Pectin, soluble fiberdextrin (SFD), or soluble corn fiber (SCF). Rats fed Pectin had a higher average daily food intake, but no differences in final body weights or rates of weight gain among treatments were observed. No differences were observed in total short-chain fatty acid (SCFA) or branched-chain fatty acid (BCFA) concentrations in the cecum and colon of rats fed either SFD or SCF. The SFD and SCF treatments increased cecal propionate and decreased butyrate concentrations compared to Control or Pectin. Pectin resulted in increased BCFA in the cecum and colon. Supplementation of SFD and SCF had no effect on cecal microbial populations compared to Control. Consumption of SFD and SCF increased total and empty cecal weight but not colon weight. Gut histomorphology was positively affected by SFD and SCF. Increased crypt depth, goblet cell numbers, and acidic mucin were observed in both the cecum and colon of rats supplemented with SFD, SCF, and Pectin. These novel, low-digestible carbohydrates appear to be beneficial in modulating indices of hindgut morphology when supplemented in the diet of the rat." As taken from Knapp BK et al. 2013. Nutrients 5(2), 396-410. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23381099>

"Water extracts of both *Morus alba* L. root bark (MBW) and *Cornus officinalis* Siebold and Zucc fruit (CFW) have traditionally been used to promote men's health in the elderly in Asia. We determined that the 12-week consumption of MBW and CFW could alleviate testosterone-deficiency syndrome and osteoarthritis (OA) symptoms in testosterone-deficient rats, and the action mechanisms were explored. Rats with bilateral orchiectomy (ORX) were fed a 45% fat diet containing either 0.5% MBW (ORX-MBW), 0.5% CFW(ORX-CFW), or 0.5% dextrin (ORX-CON). Sham-operated rats also

received 0.5% dextrin (Non-ORX-CON). After 8 weeks of treatment, all rats had an injection of monoiodoacetate (MIA) into the left knee, and they continued the same diet for the additional 4 weeks. ORX-CFW and ORX-MBW partially prevented the reduction of serum testosterone concentrations and decreased insulin resistance, compared to the ORX-CON. ORX-CFW and ORX-MBW protected against the reduction of bone mineral density (BMD) and lean body mass (LBM) compared to the ORX-CON. The limping and edema scores were lower in the order of the ORX-CON, ORX-CRF = ORX-MBW, and Non-ORX-CON ($p < 0.05$). The scores for pain behaviors, measured by weight-distribution on the OA leg and maximum running velocity on a treadmill, significantly decreased in the same order as limping scores. ORX-MBW protected against the increased expression of matrix metalloproteinase (MMP)-3 and MMP-13 and reduced the production of inflammatory markers such as TNF- α and IL-1 β , by MIA in the articular cartilage, compared to the ORX-CON ($p < 0.05$). The cartilage damage near the tidemark of the knee and proteoglycan loss was significantly less in ORX-MBW than ORX-CON. In conclusion, MBW, possibly CFW, could be effective alternative therapeutic agents for preventing osteoarthritis in testosterone-deficient elderly men.” As taken from Park S et al. 2020. *Pharmaceutics*. 12(12), 1245. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/33371279/>

7. Addiction

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

8. Burnt ingredient toxicity

This ingredient was considered as part of an overall safety assessment of ingredients added to tobacco in the manufacture of cigarettes. An expert panel of toxicologists reviewed the open literature and internal toxicology data of 5 tobacco companies to evaluate a composite list of ingredients used in the manufacture of cigarettes. The conclusion of this report was that these ingredients did not increase the inherent biological activity of tobacco cigarettes, and are considered to be acceptable under conditions of intended use (Doull et al., 1994 & 1998).

Tobacco smoke condensates from cigarettes containing Sugars (dextrin) and an additive free, reference cigarettes were tested in a battery of in vitro and/or in vivo test(s). Within the sensitivity and specificity of the bioassay(s) the activity of the condensate was not changed by the addition of Sugars (dextrin). Table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
Smoke chemistry	12	Baker et al., 2004a
In vitro genotoxicity	12	Baker et al., 2004c
	10,000	JTI KB Study Report(s)
In vitro cytotoxicity	12	Baker et al., 2004c
	10,000	JTI KB Study Report(s)
Inhalation study	12	Baker et al., 2004c
	10,000	JTI KB Study Report(s)

9. Heated/vapor emissions toxicity

Aerosol from heated tobacco stick(s) containing Dextrin 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	0.22	Labstat International Inc. (2020a) Labstat International Inc. (2021a)
In vitro genotoxicity	0.22	Labstat International Inc. (2020b) Labstat International Inc. (2021b)
In vitro cytotoxicity	0.22	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 dextrin is of uncertain persistence in the environment.

Data accessed May 2017 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 dextrin is not inherently toxic to aquatic organisms and is of low ecotoxicological concern.

Data accessed May 2017 on the OECD website: <http://webnet.oecd.org/CCRWeb/Search.aspx>

“Glucose and lipid metabolism in pancreatic islet organs is poorly characterized. In the present study, using as a model the carnivorous rainbow trout, a glucose-intolerant fish, we assessed mRNA expression levels of several genes involved in glucose and lipid metabolism (including ATP-citrate lyase; carnitine palmitoyltransferase-1 isoforms, CPT; the mitochondrial isoform of the phosphoenolpyruvate carboxykinase, mPEPCK and pyruvate kinase, PK) and glucosensing (glucose transporter type 2, Glut2; glucokinase, GK and the potassium channel, K(ATP)) in Brockmann bodies. We evaluated the response of these parameters to changes in feeding status (food deprived vs. fed fish) as well as to changes in the amount of carbohydrate (dextrin) in the diet. A general inhibition of the glycolytic (including the glucosensing marker GK) and β -oxidation pathways was found when comparing fed versus food-deprived fish. When comparing fish feeding on either low- or high-carbohydrate diets, we found that some genes related to lipid metabolism were more controlled by the feeding status than by the carbohydrate content (fatty acid synthase, CPTs). Findings are discussed in the context of pancreatic regulation of glucose and lipid metabolism in fish, and show that while trout pancreatic metabolism can partially adapt to a high-carbohydrate diet, some of the molecular actors studied seem to be poorly regulated (K(ATP)) and may contribute to the glucose intolerance observed in this species when fed high-carbohydrate diets”. As taken from Polakof S et al. (2012). J. Comp. Physiol B 182, 507-516. PubMed, 2013 available at <https://www.ncbi.nlm.nih.gov/pubmed/22203338>

10.3. Sediment toxicity

No data available to us at this time

10.4. Terrestrial toxicity

The US EPA ECOTOX database provides the following data:

Malus pumila (Paradise apple): no effect on size or fruiting or damage to the fruit at 50 g/L.

Solanum lycopersicum (tomato): decrease in growth (size) following 1 day of exposure to 2%.

Phaseolus vulgaris (bean): following 56 days of exposure, growth (biomass) increased at concentrations of 4.09 g/1.8 kg soil or 4.09 g/1.4 kg soil (statistically significant at 4.09/1.4 kg) and statistically significantly decreased at 40.90 g/1.8 kg soil or 40.90 g/1.4 kg soil.

Zea mays (corn): concentration-related, statistically significant decrease in growth (biomass) following 56 days of exposure to 4.09 or 40.90 g/1.8 kg soil; statistically significant increase at 4.09 g/1.4 kg soil and statistically significant decrease at 40.90 g/1.4 kg soil.

10.5. All other relevant types of ecotoxicity

Ecotoxicological properties of polymers of sugars are evaluated based on expert judgment as no data were found. Minimum risk is concluded based on a biodegradable structure of the compound in addition to read across to glucose for aquatic toxicity.

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that dextrin is of uncertain bioaccumulative potential in the environment.

Data accessed May 2017 on the OECD website: <http://webnet.oecd.org/CCRWeb/Search.aspx>

11. References

- AICIS (2012). Australian Government Department of Health. Australian Industrial Chemicals Introduction Scheme. Inventory Multi-Tiered Assessment and Prioritisation (IMAP) Tier I. Health record for dextrin (CAS RN 9004-53-9). Dated 7 December 2012. Available at <https://www.industrialchemicals.gov.au/chemical-information/search-assessments?assessmentcasnumber=9004-53-9>
- Argyri K et al. (2013). Dessert formulation using sucralose and dextrin affects favorably postprandial response to glucose, insulin, and C-peptide in type 2 diabetic patients. *Rev. Diabet. Stud.* 10(1), 39-48. PubMed, 2015 available at: <http://www.ncbi.nlm.nih.gov/pubmed/24172697>
- Baker et al. (2004a). The effect of tobacco ingredients on smoke chemistry. Part I: Flavourings and additives. *Food and Chemical Toxicology*, 42S, S3.
- Baker et al. (2004c). An overview of the effects of tobacco ingredients on smoke chemistry and toxicity. *Food and Chemical Toxicology*, 42S, S53.
- Baker R and Bishop L. (2005). The pyrolysis of non-volatile tobacco ingredients using a system that stimulates cigarette combustion conditions. *J. Anal. Appl. Pyrolysis* 74, 145–170.
- CCRIS (1995). Record for dextrin. CCRIS record no. 6614. Last revision date 3 July 1995 (records no longer being updated after 2011). Available at <https://www.toxinfo.io/>
- CIR (2015). Cosmetic Ingredient Review. Safety assessment of polysaccharide gums as used in cosmetics. Final report dated 22 October 2015. Available at <http://www.cir-safety.org/sites/default/files/plpogu092015final.pdf>
- CosIng. Cosmetic substances and ingredients database. Record for dextrin (CAS RN 9004-53-9). Undated, Available at <https://ec.europa.eu/growth/tools-databases/cosing/>
- COSMOS database. Integrated In Silico Models for the Prediction of Human Repeated Dose Toxicity of COSMetics to Optimise Safety. Record for dextrin (CAS RN 9004-53-9). Available at <https://ng.cosmosdb.eu/>
- CPID (undated). Consumer Product Information Database. Record for dextrin (CAS RN 9004-53-9). Available at <https://www.whatsinproducts.com/>
- Doull et al. (1994). A safety assessment of the ingredients added to tobacco in the manufacture of cigarettes. Available at <http://legacy.library.ucsf.edu/tid/thy03c00>
- Doull et al. (1998). A safety assessment of the ingredients added to tobacco in the manufacture of cigarettes. Available at <http://legacy.library.ucsf.edu/tid/wzp67e00>

- ECHA (2023). European Chemicals Agency. Classification and Labelling (C&L) Inventory database. Last updated 19 July 2023. Available at: <https://echa.europa.eu/information-on-chemicals/cl-inventory-database>
- ECHA (undated). European Chemicals Agency. Information on Chemicals. Available at: <https://echa.europa.eu/information-on-chemicals/pre-registered-substances>
- Emilien CH et al. (2020). The effect of soluble fiber dextrin on subjective and physiological markers of appetite: A randomized trial. *Nutrients* 12(11), 3341. DOI: 10.3390/nu12113341. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/33143121/>
- Farhangi MA et al. (2018). A randomized controlled trial on the efficacy of resistant dextrin, as functional food, in women with type 2 diabetes: Targeting the hypothalamic-pituitary-adrenal axis and immune system. *Clin. Nutr.* 37(4), 1216-1223. DOI: 10.1016/j.clnu.2017.06.005. PubMed, 2019 available at: <https://www.ncbi.nlm.nih.gov/pubmed/28669666>
- FDA (2013). US Food and Drug Administration. Agency Response Letter: GRAS Notice No. GRN 000436. 18 January 2013. Available at <https://wayback.archive-it.org/7993/20171031024526/https://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm338915.htm>
- FDA (2022). US Food and Drug Administration. Substances Added to Food (formerly EAFUS). Last updated 13 October 2022. Available at: <https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=FoodSubstances>
- FDA (2023). US Food and Drug Administration. Electronic Code of Federal Regulations (eCFR). Title 21. Current as of 28 March 2023. Available at <https://www.ecfr.gov/cgi-bin/ECFR?page=browse>
- Gholizadeh Shamasbi S et al. (2019). The effect of resistant dextrin as a prebiotic on metabolic parameters and androgen level in women with polycystic ovarian syndrome: a randomized, triple-blind, controlled, clinical trial. *Eur. J. Nutr.* 58(2), 629-640. DOI: 10.1007/s00394-018-1648-7. PubMed, 2019 available at: <https://www.ncbi.nlm.nih.gov/pubmed/29480399>
- Goncalves C; Ferreira MF; Santos AC; Prata MI; Geraldies CF; Martins JA; Gama FM (2010a). Studies on the biodistribution of dextrin nanoparticles. *Nanotechnology* 21(29):295103. PubMed abstract available at <http://www.ncbi.nlm.nih.gov/pubmed/20601764?dopt=AbstractPlus>
- Goncalves C; Torrado E; Martins T; Pereira P; Pedrosa J; Gama M (2010b). Dextrin nanoparticles: studies on the interaction with murine macrophages and blood clearance. *Colloids Surf B Biointerfaces*. 75(2):483-9. PubMed abstract available at <http://www.ncbi.nlm.nih.gov/pubmed/19836933?dopt=AbstractPlus>
- Haz-Map (2020). Record for dextrin (CAS RN 9004-53-9). Last updated 29 July 2020. Available at <https://haz-map.com/>
- Health Canada (2021). Natural Health Products Ingredients Database. Record for dextrin. Last modified 1 July 2021. Available at <http://webprod.hc-sc.gc.ca/nhp/bdipsn/ingredReq.do?id=140&lang=eng>
- Hobden MR et al. (2013). In vitro fermentation of NUTRIOSE(®) FB06, a wheat dextrin soluble fibre, in a continuous culture human colonic model system. *PLoS One* 8(10), e77128. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24204753>
- Hobden MR et al. (2021). Impact of dietary supplementation with resistant dextrin (NUTRIOSE®) on satiety, glycaemia, and related endpoints, in healthy adults. *Eur. J. Nutr.* Epub ahead of print. DOI: 10.1007/s00394-021-02618-9. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/34170392/>
- IFRA (undated). International Fragrance Association. IFRA Transparency List. Available at <https://ifrafragrance.org/priorities/ingredients/ifra-transparency-list>
- INCHEM (1982). WHO Food Additives Series 17. Available at <http://www.inchem.org/documents/jecfa/jecmono/v17je16.htm>

- JECFA (2021). Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Dextrins, white and yellow, roasted starch. Available at <https://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID=2553>
- JTI KB Study Report (s).
- Khan IA and Abourashed EA (2010). Leung's Encyclopedia of Common Natural Ingredients used in Food, Drugs, and Cosmetics. Third Edition. John Wiley & Sons, Inc., Hoboken, New Jersey.
- Kimura T (2013). The regulatory effects of resistant starch on glycaemic response in obese dogs. Arch. Anim. Nutr. 67(6), 503-9. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24228912>
- Knapp BK et al. (2013). Soluble fiber dextrin and soluble corn fiber supplementation modify indices of health in cecum and colon of Sprague-Dawley rats. Nutrients 5(2), 396-410. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23381099>
- Labstat International Inc. (2020a) Characterization of Heat-not-Burn Emissions. Analytical Test Report(s).
- Labstat International Inc. (2020b) Determination of Mutagenic Response (Ames), Cytotoxic Response (NRU) and Genotoxic Response (ivMN) of Mainstream Aerosol Total Particulate Matter (TPM) and Mainstream Gas Vapor Phase (GVP) of Heat-not-burn Products. Biological Activity Test Report(s).
- Labstat International Inc. (2021a). Characterization of Heat-not-Burn Emissions. Analytical Test Report(s).
- Labstat International Inc. (2021b). Determination of Mutagenic Response (Ames), Cytotoxic Response (NRU) and Genotoxic Response (ivMN) of Mainstream Aerosol Total Particulate Matter (TPM) and Mainstream Gas Vapor Phase (GVP) of Heat-not-burn Products. Biological Activity Test Report(s).
- Lefranc-Millot C et al. (2012). Impact of a resistant dextrin on intestinal ecology: how altering the digestive ecosystem with NUTRIOSE®, a soluble fibre with prebiotic properties, may be beneficial for health. J. Int. Med. Res. 40, 211-224. PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/22429361>
- Liu Qi and Laskowski JS (1989). The interactions between dextrin and metal hydroxides in aqueous solutions, Journal of Colloid and Interface Science, Volume 130, Issue 1, June 1989, Pages 101-111. Science Direct available at <http://www.sciencedirect.com/>
- Marteau P; Guerin-Deremaux L; Wils D; Cazaubiel M; Housez B (2011). Short-term digestive tolerance of high-dose of NUTRIOSE®FB10 in adult. Int J Food Sci Nutr. 62(2):97-101. PubMed abstract available at <http://www.ncbi.nlm.nih.gov/pubmed/20849371?dopt=AbstractPlus>
- Nader N et al. (2014). Effects of fiber supplementation on glycemic excursions and incidence of hypoglycemia in children with type 1 diabetes. Int. J. Pediatr. Endocrinol. 2014(1), 13. PubMed, 2015 available at: <http://www.ncbi.nlm.nih.gov/pubmed/25024710>
- OECD. Organisation for Economic Cooperation and Development. The Global Portal to Information on Chemical Substances (eChemPortal). Dextrin (CAS RN 9004-53-9). Accessed May 2017. Available at: <http://webnet.oecd.org/CCRWeb/Search.aspx>
- Park S et al. (2020). Aqueous extracts of Morus alba root bark and Cornus officinalis fruit protect against osteoarthritis symptoms in testosterone-deficient and osteoarthritis-induced rats. Pharmaceutics 12(12), 1245. DOI: 10.3390/pharmaceutics12121245. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/33371279/>
- Polakof S et al. (2012). Glucose and lipid metabolism in the pancreas of rainbow trout is regulated at the molecular level by nutritional status and carbohydrate intake. J. Comp. Physiol. B 182, 507-516. PubMed, 2013 available at <https://www.ncbi.nlm.nih.gov/pubmed/22203338>

- Poulsen M et al. (2001). The influence of simple sugars and starch given during pre- or post-initiation on aberrant crypt foci in rat colon Cancer Lett. 2001, Jun 26; 167(2):135-43. PubMed, 2010 available at <http://www.ncbi.nlm.nih.gov/pubmed/11369133>
- PubChem (2023). Record for dextrin (CAS RN 9004-53-9). Created 8 August 2005. Last modified 17 July 2023. Available at <https://pubchem.ncbi.nlm.nih.gov/compound/62698>
- Shimizu Y et al. (2021). Glucagon-like peptide-1 response to whey protein is less diminished by dipeptidyl peptidase-4 in comparison with responses to dextrin, a lipid and casein in rats. Br. J. Nutr. 125(4), 398-407. DOI: 10.1017/S0007114520002834. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/32713353/>
- Slavin J (2013). Fiber and prebiotics: mechanisms and health benefits. Nutrients 5(4), 1417-35. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23609775>
- Slizewska K (2013). The citric acid-modified, enzyme-resistant dextrin from potato starch as a potential prebiotic. Acta Biochim. Pol. 60(4), 671-5. PubMed, 2015 available at: <http://www.ncbi.nlm.nih.gov/pubmed/24432315>
- US EPA (2023a). Safer Chemical Ingredients List. Last updated 16 April 2023. Available at <https://www.epa.gov/saferchoice/safer-ingredients>
- US EPA (2023b). US Environmental Protection Agency. Electronic Code of Federal Regulations (eCFR). Title 40. Current as of 15 July 2023. Available at <https://www.ecfr.gov/cgi-bin/ECFR?page=browse>
- US EPA 2020 CDR Full Exempt list (Chemical Data Reporting Rule). Available at https://sor.epa.gov/sor_internet/registry/substreg/LandingPage.do
- US EPA 2020 CDR list (Chemical Data Reporting Rule). Available at https://sor.epa.gov/sor_internet/registry/substreg/LandingPage.do
- US EPA ECOTOX database. Record for dextrin (CAS RN 9004-53-9). Accessed May 2017. Available at http://cfpub.epa.gov/ecotox/quick_query.htm
- US EPA InertFinder Database (2023). Record for ethanol, 2,2'-[1,2-ethanediylbis(oxy)]bis-, dibenzoate. Last updated 24 May 2023 Available at <https://iaspub.epa.gov/apex/pesticides/f?p=INERTFINDER:1:0::NO:1::>
- US EPA Office of Pesticide Programs (undated). Record for dextrin (CAS RN 9004-53-9). Available at https://iaspub.epa.gov/apex/pesticides/f?p=CHEMICALSEARCH:3::::1,3,31,7,12,25:P3_XCHEMICAL_ID:2061
- US EPA TSCA (Toxic Substances Control Act) inventory. Available at https://sor.epa.gov/sor_internet/registry/substreg/LandingPage.do
- Valcheva R et al. (2015) Soluble Dextrin Fibers Alter the Intestinal Microbiota and Reduce Proinflammatory Cytokine Secretion in Male IL-10-Deficient Mice. J. Nutr. 145(9), 2060-6. PubMed, 2016 available at <https://pubmed.ncbi.nlm.nih.gov/26180249/>
- Wandrey et al. (2010). Encapsulation Technologies for Active Food Ingredients and Food Processing. Chapter 3. Materials for Encapsulation. Springer ScienceBusiness Media, LLC 2010. 978-1-4419-1008-0_3. Available at https://books.google.co.uk/books?id=wi3GFjTn5gcC&printsec=copyright&redir_esc=y
- Zhai W; Bennett LW; Gerard PD; Pulikanti R; Peebles ED (2011a). Effects of in ovo injection of carbohydrates on somatic characteristics and liver nutrient profiles of broiler embryos and hatchlings. Poult Sci. 90(12):2681-8. PubMed abstract available at <http://www.ncbi.nlm.nih.gov/pubmed/22080004?dopt=AbstractPlus>
- Zhai W; Gerard PD; Pulikanti R; Peebles ED (2011b). Effects of in ovo injection of carbohydrates on embryonic metabolism, hatchability, and subsequent somatic characteristics of broiler hatchlings. Poult Sci. 90(10):2134-43. PubMed abstract available at <http://www.ncbi.nlm.nih.gov/pubmed/21933993?dopt=AbstractPlus>
- Zhai W; Rowe DE; Peebles ED (2011c). Effects of commercial in ovo injection of carbohydrates on broiler embryogenesis. Poult Sci. 90(6):1295-301. PubMed abstract available at <http://www.ncbi.nlm.nih.gov/pubmed/21597071?dopt=AbstractPlus>

12. Other information

- Colantonio AG et al. (2020). The effects of prebiotics and substances with prebiotic properties on metabolic and inflammatory biomarkers in individuals with Type 2 Diabetes mellitus: A systematic review. J. Acad. Nutr. Diet 120(4), 587-607. DOI: 10.1016/j.jand.2018.12.013. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/30827823/>
- Devasia S et al. (2019). A double blind, randomised, four arm clinical study to evaluate the safety, efficacy and tolerability of collagen peptide as a nutraceutical therapy in the management of Type II Diabetes mellitus. J. Diabetes Metab. 11(1), 839. DOI: 10.35248/2155-6156.19.10.839. Available at <https://www.longdom.org/open-access/a-double-blind-randomised-four-arm-clinical-study-to-evaluate-the-safety-efficacy-and-tolerability-of-collagen-peptide-a.pdf>
- Farhangi MA et al. (2019). Prebiotic supplementation modulates advanced glycation end-products (AGEs), soluble receptor for AGEs (sRAGE), and cardiometabolic risk factors through improving metabolic endotoxemia: A randomized-controlled clinical trial. Eur. J. Nutr. Epub ahead of print. DOI: 10.1177/1534735417721014. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/31728681/>
- Oshima S et al. (2019). Combined supplementation with glycine and tryptophan reduces purine-induced serum uric acid elevation by accelerating urinary uric acid excretion: A randomized, single-blind, placebo-controlled, crossover study. Nutrients 11(11), 2562. DOI: 10.3390/nu11112562. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/31652875/>

13. Last audited

July 2023

DEXTRINS

Explanation

This substance was evaluated previously for an ADI for man by the Joint FAO/WHO Expert Committee on Food Additives in 1969 and 1974 (see Annex I, Refs. 19 and 29). Toxicological monographs were published in 1969 and 1974 (see Annex I, Refs. 20 and 30).

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

Introduction

White dextrins are prepared by heating dry starch in the presence of an acid at a temperature generally below 150°C. White dextrins may also be obtained by further continuing the acid process for making thin boiling starches. Because of the nature of the application as well as their flavour, their use in food is restricted. Dextrins are a stage in the normal digestion of starch occurring in the human gastrointestinal tract. They represent a broad range of products with considerably smaller molecular size than native starch.

Yellow dextrins are prepared in a similar manner but at a higher temperature and using less acid. Apart from depolymerization, a good deal of internal rearrangement occurs with formation of highly branched molecules. These materials are used in foods in limited quantities as adjuvants in flavour encapsulation and similar minor uses.

BIOLOGICAL DATA

BIOCHEMICAL ASPECTS

Absorption and metabolism

Dextrins and their parent starches were fed to groups of 6 weanling male rats (strain unspecified), initial weight 45-60 g, at a level of 60 g/kg bw for 21 to 28 days. Diets contained 18.8% casein. The digestibility of wheat dextrin was somewhat lower than that of wheat starch. The potato dextrin gave a higher body weight gain and digestibility coefficient than the parent starch (Booher et al., 1951).

In a study on the effect of type of dietary carbohydrate on B-vitamin synthesis in the digestive tracts of rats, groups of 17 to 44 male and female rats (strain unspecified), 21 days of age were placed on test diets containing 18% protein (casein), 71%

carbohydrate, and 3% butterfat, cod-liver oil and salt mixture. The carbohydrate sources used included cornstarch, corn dextrin, glucose, lactose and sucrose. Animals on all diets, without supplemental B-vitamins but with access to their faeces, showed low or declining growth rates after 2 weeks except for the group fed the dextrin diet. Growth rates in all groups were increased after receiving faeces of the dextrin-fed group. Rats receiving the dextrin diet had enlarged caeca at the conclusion of the study. Caecectomized rats with access to their faeces lost weight when fed a dextrin diet; supplementation with baker's yeast resulted in weight gain. It was concluded that the peculiar property of corn dextrin was not due to retained B-vitamins, but rather to the formation of these vitamins in the lower part of the digestive tract of the rat as a result of incomplete digestion of this particular carbohydrate (Guerrant et al., 1935).

Fournier (1959) studied the effect of various dietary carbohydrate sources on calcium retention, serum calcium levels, and caecal size in the rat. Wistar rats (sex unspecified) weighing 62-74 g, were fed a low calcium diet (50 mg Ca/100 g diet) for 18 days after which groups of 6 rats were placed on diets containing 15% casein, 1.5% calcium carbonate and 45-70% experimental carbohydrate (starch, dextrin, caramel or glucose) plus cereal grain to bring total carbohydrate to about 70%. Rats received an estimate of 46 g dextrin per kg body weight. Calcium balance was determined during the third to fifth day; after 10 days the rats were sacrificed and serum calcium determined. Caecal enlargement observations were made after 2 weeks of feeding a diet containing 75% of the experimental carbohydrate source, 12% casein, 8% peanut oil and 3% salts. Calcium intake was approximately the same for all diets, but calcium retention for the dextrin and caramel diets was nearly double that for the starch and glucose diets. Serum calcium levels also were greater for the dextrin and caramel diets. Dry caecal weights of rats fed dextrin and caramel were more than double those fed the starch and glucose diets. The author postulated that dextrin and caramel were less easily metabolized than their parent substances, starch and glucose, and that this property contributed to the effects observed.

¹⁴C-labelled beta-cyclodextrin homogenized in aqueous dextran was administered to 5 Wistar plus Long Evans F₁ hybrid male rats weighing about 200 g each. Individual animals received doses corresponding to their body weight through an oesophageal cannula. Three control animals received ¹⁴C-labelled glucose. Blood levels of

the compounds were measured at intervals up to 97 hours from tail vein samples. At 7, 12 and 24 hours after administration, selected animals were decapitated, and radioactivity was measured in stomach, small intestine and colon. In the case of the cyclodextrin, a maximum of only 5% of the administered activity could be found in blood even after 10 hours; after 96 hours the same residual radioactivity was found in the blood with both glucose and β -cyclodextrin. This study

suggests that β -cyclodextrin cannot be absorbed either from the stomach or the small intestine; only the labelled open-chain dextrans and glucose formed from cyclodextrin by amylase action were absorbed (Szejtli et al., 1980).

TOXICOLOGICAL STUDIES

Special studies on nephrosis in the rat kidney due to alpha- and beta-cyclodextrin

Groups of 5 male and 5 female Sprague-Dawley rats weighing 150 to 160 g were administered cyclodextrins intravenously. The i.v. LD₅₀ for rats was determined to be 0.79-1.0 g/kg bw with a close relationship between the acute toxicity and the nephrotoxic dose (Frank et al., 1976).

Groups of 4 100-124 g Sprague-Dawley rats received single s.c. doses of cyclodextrins of 0.225, 0.45 or 0.90 g/kg and were killed 12, 24, 48 or 96 hours later. Controls received saline injections. Kidneys were sectioned for light microscopy and histopathological observations (Frank et al., 1976).

In another experiment, groups of 4 100-125 g Sprague-Dawley rats were given 1, 2, 3, 4 or 7 daily s.c. injections of cyclodextrins at 0.225, 0.45, 0.675, 0.90, 0.1 or 1.0 g/kg bw. Controls received saline injections. Rats were killed 24 hours after the last injection and kidneys were sectioned for histopathological observations (Frank et al., 1976).

Renal toxicity due to the cyclodextrins was shown to result from a series of alterations in the vacuolar organelles of the proximal convoluted tubules. Intracellular concentration of toxin via the lysosomal pathway resulted in a change of the physiological function of the proximal tubule which ultimately leads to cell death (Frank et al., 1976).

Short-term studies

Rat

Groups of 10 male Wistar rats received diets with 6 or 15% protein from casein and 77 or 66% carbohydrate (75 and 65 g carbohydrate/kg bw). After 28 days, protein efficiency and weight gain/g of dry food were significantly lower for corn dextrin than for cornstarch, but were greater or equal to values for dextrose. Corn dextrin diets caused no unusual effect on liver weight or liver fat content; however, rats receiving corn dextrin exhibited a slight diarrhoea and caecal enlargement to about twice that in rats fed unmodified cornstarch (Reussner et al., (1963).

Groups of 6 Sprague-Dawley male weanling rats (initial weight 40-50 g), were fed for periods of 2-12 weeks on diets containing approximately 80 g dextrin/kg bw; diets contained 81% carbohydrate, 9% casein and 5% corn oil. Rates of gain with the dextrans over a 4-week period were about 15% less than that for autoclaved cornstarch; the latter weight was about double when the carbohydrate source was glucose or sucrose. Liver fat deposition was less for one of the dextrans, cornstarch or glucose, than for sucrose as the carbohydrate source. Liver fat deposition values were not reported for the other dextrin (Harper et al., 1953).

Groups of 5 or 10 male weanling Sprague-Dawley or Osborne-Mendel rats, weighing initially 40-50 g, were fed diets containing about 80 g carbohydrate/kg bw; diets consisted of 87% carbohydrate, 9% casein, 3% gelatin and 3% corn oil. Weight gain over a 4-week period with niacin supplementation was the same with either dextrin, starch or glucose as carbohydrate source, without niacin supplementation, growth rate decreased about 40% for starch and dextrin as the carbohydrate source, as compared to 60% decrease with glucose as the carbohydrate source, suggesting a lesser niacin requirement with starch and dextrin as carbohydrate source (Hundley, 1949).

Long-term studies

Groups of 9 male Sprague-Dawley rats (2 months of age) were fed diets with different sources of carbohydrate for a 20-month period. Diets consisted of rat chow mixed with 20%, by weight, of the various carbohydrate sources, including dextrin, sucrose, and dextrose. Rats received approximately 10 g experimental carbohydrate/kg bw. Protein efficiency ratios calculated after 6 months feeding were nearly equal for the dextrin, dextrose and sucrose diets and significantly higher than for the basal rat chow diet. Weight gain after 20 months of feeding was about 5% less for dextrin than for dextrose or sucrose but about 5% more than on the basal rat chow (Cohen et al., 1967).

Comments

These substances are regarded as identical to the intermediates formed in the normal digestion of starch and normal constituents of food.

EVALUATION

Estimate of acceptable daily intake for man

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 an acceptable daily intake (ADI) in mg/kg bw is not deemed necessary.

REFERENCES

- Booher, L. E., Behan, I. & McMeans, E. (1951) Biological utilization of unmodified and modified food starches, J. Nutr., 45, 75-95
- Cohen, L., Perkin, E. G. & Dobrilovic, L. (1967) The manifold effects of different dietary carbohydrates, Progr. Biochem. Pharmacol., 2, 182-202
- Fournier, P. (1959) Le caramel et la dextrine préparés par action de la chaleur sèche sur le glucose et l'amidon possèdent les qualités physiologiques des composés de structure, C. R. Acad. Sci., 248, 3744-3746
- Frank, D. W., Gray, J. E. & Weaver, R. N. (1976) Cyclodextrin nephrosis in the rat, Am. J. Pathol., 83(2), 367-382
- Guerrant, N. B., Dutcher, R. A. & Tomey, L. F. (1935) The effect of the type of carbohydrate on the synthesis of B vitamins in the digestive tract of the rat, J. Biol. Chem., 110, 223-243
- Harper, A. E. et al. (1953) Influence of various carbohydrates on the utilization of low protein rations by the white rat, J. Nutr., 51, 523-537
- Hundley, J. M. (1949) Influence of fructose and other carbohydrates on

the niacin requirement of the rat, J. Biol. Chem., 181, 1-9

Reussner, G., Jr, Andros, J. & Thiessen, R., Jr (1963) Studies on the utilization of various starches and sugars in the rat, J. Nutr., 80, 291-298

Szejtli, J., Gerl'oczy, A. & F'Onagy, A. (1980) Intestinal absorption of the ¹⁴C-labelled beta-cyclodextrin in rats, Arzneim., 30(5), 808-810

See Also:

Toxicological Abbreviations

<i>Substance</i>	<i>ID Code</i>	<i>Rpt No.</i>	<i>Year</i>	<i>Conclusion*</i>	<i>21 CFR Section</i>
Dextrins	9004-53-9	75	1975	1	184.1277

SCOGS Opinion:

The dextrins covered by this report are those produced by the dry heating of unmodified starch under the range of conditions specified in the body of the report as representative of commercial practice for this class of products. Included are the white dextrins, yellow or canary dextrins and the British gums. The dextrins are similar to their parent starches in that they are composed principally of α -D-anhydroglucose units joined through 1,4-linkages; they differ in that dextrinization reduces the molecular weight and, particularly in case of the yellow or canary dextrins and the British gums, increases branching in the molecules. Dextrinization slightly reduces the digestibility of corn and wheat starch, probably attributable to the more highly branched structure of the dextrins.

Animal feeding studies have shown dextrins to be digested and metabolized to a limited degree without toxic effects when fed at levels many times greater than those present from use of these products as a direct food additive, or at levels that are orders of magnitude greater than might occur by migration from food packaging materials containing dextrins. The Select Committee concludes that: There is no evidence in the available information on dextrins and corn dextrin that demonstrates or suggests reasonable grounds to suspect, a hazard to the public when they are used at levels that are now current or that might be reasonably expected in the future.

** denotes Type of Conclusion 1, 2, 3, 4, or 5. Definitions of conclusion types can be found at the end of this report..*

SCIENTIFIC OPINION

Scientific Opinion on the substantiation of a health claim related to “non-fermentable” carbohydrates and maintenance of tooth mineralisation by decreasing tooth demineralisation pursuant to Article 13(5) of Regulation (EC) No 1924/2006¹

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)^{2,3}

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Following an application from Roquette Frères, submitted for authorisation of a health claim pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of France, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on the scientific substantiation of a health claim related to Nutriose[®] which should replace “fermentable carbohydrates” in foods or beverages in order to obtain the claimed effect, i.e. maintenance of tooth mineralisation by reducing tooth demineralisation. From the information provided, the Panel noted that the main characteristic of carbohydrates which is relevant to the claimed effect is the rate and amount of acid production resulting from their fermentation by saccharolytic bacteria in the oral cavity. This Opinion applies to “non-fermentable” carbohydrates, which should replace “fermentable” carbohydrates in foods or beverages in order to obtain the claimed effect. The Panel considers that maintaining tooth mineralisation by reducing tooth demineralisation resulting from acid production in plaque caused by the fermentation of carbohydrates is a beneficial physiological effect. The Panel concludes that a cause and effect relationship has been established between the consumption of foods/beverages containing “fermentable” carbohydrates at an exposure frequency of four or more times daily and an increased tooth demineralisation, and that the consumption of foods/beverages containing “non-fermentable” carbohydrates instead of “fermentable” carbohydrates may maintain tooth mineralisation by decreasing tooth demineralisation. In order to bear the claim, “fermentable” carbohydrates should be replaced in foods or beverages by “non-fermentable” carbohydrates, so that consumption of such foods or beverages does not lower plaque pH below 5.7 during and up to 30 minutes after consumption, and does not lead to dental erosion.

© European Food Safety Authority, 2013

¹ On request from the Competent Authority of France following an application by Roquette Frères, Question No EFSA-Q-2013-00040, adopted on 10 July 2013.

² Panel members: Carlo Agostoni, Roberto Berni Canani, Susan Fairweather-Tait, Marina Heinonen, Hannu Korhonen, Sébastien La Vieille, Rosangela Marchelli, Ambroise Martin, Androniki Naska, Monika Neuhäuser-Berthold, Grażyna Nowicka, Yolanda Sanz, Alfonso Siani, Anders Sjödin, Martin Stern, Sean (J.J.) Strain, Inge Tetens, Daniel Tomé, Dominique Turck and Hans Verhagen. Correspondence: nda@efsa.europa.eu

³ Acknowledgement: The Panel wishes to thank the members of the Working Group on Claims: Carlo Agostoni, Jean-Louis Bresson, Susan Fairweather-Tait, Marina Heinonen, Ambroise Martin, Hildegard Przyrembel, Yolanda Sanz, Alfonso Siani, Anders Sjödin, Sean (J.J.) Strain, Inge Tetens, Hendrik Van Loveren, Hans Verhagen and Peter Willatts for the preparatory work on this scientific opinion.

Suggested citation: EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2013. Scientific Opinion on the substantiation of a health claim related to “non-fermentable” carbohydrates and maintenance of tooth mineralisation by decreasing tooth demineralisation pursuant to Article 13(5) of Regulation (EC) No 1924/2006. EFSA Journal 2013;11(7):3329, 13 pp. doi:10.2903/j.efsa.2013.3329

Available online: www.efsa.europa.eu/efsajournal

KEY WORDS

Nutriose[®], dietary fibre, non-fermentable carbohydrates, tooth mineralisation, tooth demineralisation, enamel, health claims

SUMMARY

Following an application from Roquette Frères, submitted for authorisation of a health claim pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of France, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on the scientific substantiation of a health claim related to Nutriose[®] and maintenance of tooth mineralisation by reducing tooth demineralisation.

The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence. The application included a request for the protection of proprietary data.

The applicant stated that the food that is the subject of the health claim is Nutriose[®], which should replace “fermentable carbohydrates” (i.e. sugars and starches) in foods or beverages in order to obtain the claimed effect (i.e. “maintenance of tooth mineralisation by decreasing tooth demineralisation”).

From the information provided, the Panel notes that the main characteristic of carbohydrates which is relevant to the claimed effect is the rate and amount of acid production resulting from their fermentation by saccharolytic bacteria in the oral cavity, and that this characteristic can be measured *in vivo* or *in situ* by plaque pH-telemetry under standard conditions.

In this context, and for the purpose of this Opinion, “fermentable” carbohydrates are defined as carbohydrates or carbohydrate mixtures as consumed in foods or beverages that lower plaque pH, as determined *in vivo* or *in situ* by plaque pH telemetry tests, below a conservative value of 5.7 by bacterial fermentation during and up to 30 minutes after consumption. Also, for the purpose of this Opinion, the term “non-fermentable” carbohydrates denotes carbohydrates or carbohydrate mixtures as consumed in foods or beverages that do not lower plaque pH below 5.7 under the same test conditions.

This Opinion applies to “non-fermentable” carbohydrates, which should replace “fermentable” carbohydrates in foods or beverages in order to obtain the claimed effect. The Panel notes that “non-fermentable” carbohydrates which have a neutral taste cannot substitute for the sweet taste of sugar.

The Panel considers that the food constituent, “non-fermentable” carbohydrates, which is the subject of the health claim, and the food constituent (i.e. “fermentable” carbohydrates) that “non-fermentable” carbohydrates should replace in foods or drinks are sufficiently characterised in relation to the claimed effect.

The claimed effect is “maintenance of tooth mineralisation by decreasing tooth demineralisation”. The target population proposed by the applicant is the general population. The Panel considers that maintaining tooth mineralisation by reducing tooth demineralisation resulting from acid production in plaque caused by the fermentation of carbohydrates is a beneficial physiological effect.

The evidence available from consensus opinions, reports from authoritative bodies and reviews shows that an increased risk of dental caries in children is associated with a high frequency of intake of “cariogenic” sugars, and that frequent consumption of sweets, confectionery products and sugar-containing drinks is associated with a higher risk of caries. Foods rich in starch, especially when the starch molecule is easily available to degradation by amylase, may also contribute to the higher risk of caries.

The evidence available from consensus opinions, reports from authoritative bodies and reviews indicates that the decrease in pH in plaque as a consequence of metabolic acid production by saccharolytic bacteria when exposed to fermentable carbohydrates (i.e. sugars and starches) may promote demineralisation and prevent remineralisation of hydroxyapatite crystals. Tooth hydroxyapatite crystals are very resistant to dissolution at neutral pH, but their solubility drastically

increases as pH drops. The Panel considers that foods lowering plaque pH, as determined *in vivo* or *in situ* by pH telemetry, below a conservative value of 5.7 by bacterial fermentation during and up to 30 minutes after consumption may promote demineralisation and prevent remineralisation of hydroxyapatite crystals.

A claim on a number of sugar replacers and maintenance of tooth mineralisation by decreasing tooth demineralisation has already been assessed with a favourable outcome. The Panel considers that the scientific substantiation and proposed conditions of use for the above-mentioned claim on sugar replacers also apply to “non-fermentable” carbohydrates, which should replace “fermentable carbohydrates” in foods and beverages in order to obtain the claimed effect.

The Panel concludes that a cause and effect relationship has been established between the consumption of foods/beverages containing “fermentable” carbohydrates at an exposure frequency of four or more times daily and an increased tooth demineralisation, and that the consumption of foods/beverages containing “non-fermentable” carbohydrates instead of “fermentable” carbohydrates may maintain tooth mineralisation by decreasing tooth demineralisation, provided that such foods/beverages do not lead to dental erosion.

The Panel considers that in order to bear the claim, “fermentable” carbohydrates should be replaced in foods or beverages by “non-fermentable” carbohydrates, so that consumption of such foods or beverages does not lower plaque pH below 5.7 during and up to 30 minutes after consumption, and does not lead to dental erosion.

TABLE OF CONTENTS

Abstract	1
Summary	3
Table of contents	5
Background	6
Terms of reference	6
EFSA Disclaimer.....	7
Information provided by the applicant	8
Assessment	8
1. Characterisation of the food/constituent	8
2. Relevance of the claimed effect to human health.....	10
3. Scientific substantiation of the claimed effect	10
4. Panel’s comments on the proposed wording.....	11
5. Conditions and restrictions of use	11
Conclusions	11
Documentation provided to EFSA	12
References	12

BACKGROUND

Regulation (EC) No 1924/2006⁴ harmonises the provisions that relate to nutrition and health claims, and establishes rules governing the Community authorisation of health claims made on foods. As a rule, health claims are prohibited unless they comply with the general and specific requirements of this Regulation, are authorised in accordance with this Regulation, and are included in the lists of authorised claims provided for in Articles 13 and 14 thereof. In particular, Article 13(5) of this Regulation lays down provisions for the addition of claims (other than those referring to the reduction of disease risk and to children’s development and health) which are based on newly developed scientific evidence, or which include a request for the protection of proprietary data, to the Community list of permitted claims referred to in Article 13(3).

According to Article 18 of this Regulation, an application for inclusion in the Community list of permitted claims referred to in Article 13(3) shall be submitted by the applicant to the national competent authority of a Member State, which will make the application and any supplementary information supplied by the applicant available to the European Food Safety Authority (EFSA).

STEPS TAKEN BY EFSA

- The application was received on 14/01/2013.
- The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence. The application included a request for the protection of proprietary data.
- On 14/02/2013, during the validation process of the application, EFSA sent a request to the applicant to provide missing information.
- On 19/03/2013, EFSA received the missing information as submitted by the applicant.
- The scientific evaluation procedure started on 08/04/2013.
- On 25/04/2013, the Working Group on Claims of the NDA Panel agreed on a list of questions for the applicant to provide additional information to accompany the application. The clock was stopped on 02/05/2013 and restarted on 17/05/2013, in compliance with Article 18(3) of Regulation (EC) No 1924/2006.
- On 21/05/2013, EFSA received the requested information (which was made available to EFSA in electronic format on 17/05/2013).
- During its meeting on 10/07/2013, the NDA Panel, having evaluated the data submitted, adopted an opinion on the scientific substantiation of a health claim related to “non-fermentable” carbohydrates and maintenance of tooth mineralisation by reducing tooth demineralisation.

TERMS OF REFERENCE

EFSA is requested to evaluate the scientific data submitted by the applicant in accordance with Article 16(3) of Regulation (EC) No 1924/2006. On the basis of that evaluation, EFSA will issue an opinion on the scientific substantiation of a health claim related to: “non-fermentable” carbohydrates and maintenance of tooth mineralisation by reducing tooth demineralisation.

⁴ Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. OJ L 404, 30.12.2006, p. 9–25.

EFSA DISCLAIMER

The present opinion does not constitute, and cannot be construed as, an authorisation for the marketing of “non-fermentable” carbohydrates, a positive assessment of their safety, nor a decision on whether “non-fermentable” carbohydrates are, or are not, classified as a foodstuff. It should be noted that such an assessment is not foreseen in the framework of Regulation (EC) No 1924/2006.

It should also be highlighted that the scope, the proposed wording of the claim, and the conditions of use as proposed by the applicant may be subject to changes, pending the outcome of the authorisation procedure foreseen in Article 18(4) of Regulation (EC) No 1924/2006.

INFORMATION PROVIDED BY THE APPLICANT

Applicant’s name and address: Roquettes Frères, FR-62136 Lestrem, France.

The application includes a request for the protection of proprietary data for three unpublished studies (Imfeld, 2002, 2010a, 2010b), in accordance with Article 21 of Regulation (EC) No 1924/2006.

Food/constituent as stated by the applicant

According to the applicant, the food/constituent is Nutriose[®], which is derived from wheat or maize, and which contains on average 85 % resistant dextrin.

Health relationship as claimed by the applicant

According to the applicant, a decrease of pH in plaque as a consequence of acid production by metabolically active saccharolytic bacteria exposed to fermentable carbohydrates (i.e. sugars and starches) may promote demineralisation and prevent remineralisation of the hydroxyapatite crystals in tooth enamel.

The intake of Nutriose[®] is claimed by the applicant not to lower the pH in plaque to a critical level (typically around pH 5.5) associated with tooth enamel dissolution.

Hence, according to the applicant, the consumption of food/drinks containing Nutriose[®] instead of sugar may help maintain tooth mineralisation by decreasing tooth demineralisation.

Wording of the health claim as proposed by the applicant

The applicant has proposed the following wording for the health claim: “Frequent consumption of sugars contributes to tooth demineralisation. Consumption of food/drinks containing Nutriose[®] instead of sugar may help maintain tooth mineralisation by decreasing tooth demineralisation”.

Specific conditions of use as proposed by the applicant

The applicant has proposed to use Nutriose[®] as a substitute for “sugar”, sucrose or starch in foods and beverages. Up to 45 g (for adults) or 20 g (for children) of Nutriose[®] may be consumed per day. The target population is the general population.

ASSESSMENT

1. Characterisation of the food/constituent

The applicant states that the food that is the subject of the health claim is Nutriose[®], which should replace “fermentable carbohydrates” (i.e. sugars and starches) in foods or beverages in order to obtain the claimed effect (i.e. “maintenance of tooth mineralisation by decreasing tooth demineralisation”).

Nutriose[®] is produced from wheat or maize starch by dry roasting under acidic conditions (i.e. dextrinisation). The ensuing dextrin is treated enzymatically with α -amylase and then purified by chromatographic partitioning in order to minimise the content of mono- and disaccharides. During the manufacturing process, linear and/or branched glucosidic bonds (i.e. α -1,2; α -1,3; β -1,2; β -1,3; β -1,4; β -1,6) are formed, which render dextrins resistant to digestion by salivary and pancreatic amylases. The final product contains about 85 % resistant dextrin, with an average degree of polymerization

from 12 to 25, and less than 0.5 % of mono- and disaccharides. An overview of the manufacturing process, batch to batch variability, stability and microbiological analysis was provided.

The taste of Nutriose[®] is described by the applicant as neutral. According to the applicant, Nutriose[®] can be used to replace “fermentable carbohydrates” in a variety of foods, such as cereals, dairy products, confectionery and beverages.

Tooth hydroxyapatite crystals are very resistant to dissolution at neutral pH, but their solubility drastically increases as pH drops. Typically, the critical pH for dental enamel is around 5.5 (FDA, 1996; EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2011). The applicant claims that the decrease in pH in plaque as a consequence of metabolic acid production by saccharolytic bacteria when exposed to fermentable carbohydrates (i.e. sugars and starches) may promote demineralisation and prevent remineralisation of the hydroxyapatite crystals. The applicant also claims that the intake of Nutriose[®] does not lower the pH in plaque to a critical level (typically around pH 5.5) associated with tooth enamel dissolution, as shown in three provided studies (Imfeld, 2002, 2010a, 2010b, unpublished, claimed as proprietary by the applicant) which included 18 plaque pH-telemetry tests carried out with 10 % solutions of Nutriose[®].

From the information provided, the Panel notes that the main characteristic of carbohydrates which is relevant to the claimed effect is the rate and amount of acid production resulting from their fermentation by saccharolytic bacteria in the oral cavity, and that this characteristic can be measured *in vivo* or *in situ* by plaque pH-telemetry under standard conditions. The Panel also notes that the rate, and the extent to which, carbohydrates are fermented by saccharolytic bacteria in the oral cavity depends on the structure of carbohydrates (e.g. chain length and type of glycosidic bonds), on other characteristics (e.g. accessibility of carbohydrates to bacterial/human enzymes, and other food constituents present) of the food or beverage in which carbohydrates are consumed, and to individual factors (e.g. oral microbiota) (Moynihan, 1998).

In this context, and for the purpose of this Opinion, “fermentable” carbohydrates are defined as carbohydrates or carbohydrate mixtures as consumed in foods or beverages that lower plaque pH, as determined *in vivo* or *in situ* by plaque pH telemetry tests, below a conservative value of 5.7 by bacterial fermentation during and up to 30 minutes after consumption (FDA, 1996). Also, for the purpose of this Opinion, the term “non-fermentable” carbohydrates denotes carbohydrates or carbohydrate mixtures as consumed in foods or beverages that do not lower plaque pH below 5.7 under the same test conditions.

EFSA noted that replacing “fermentable” carbohydrates with any “non-fermentable” carbohydrate would contribute to the claimed effect, and therefore requested the applicant to indicate which characteristics or properties of Nutriose[®] make Nutriose[®] unique as compared to other “non-fermentable” carbohydrates in relation to the claimed effect, and which could justify a product-specific claim. In response, the applicant indicated that Nutriose[®] is different from other non-viscous fibres on the market due to its low content of monosaccharides, disaccharides and low molecular weight oligosaccharides; that non-viscous soluble dietary fibres are highly fermented in the colon; and that non-viscous soluble dietary fibres other than Nutriose[®] are also fermented in the mouth because they contain disaccharides and monosaccharides at levels that promote fermentation by saccharolytic bacteria.

The Panel notes that the characteristic of Nutriose[®] which is relevant to the claimed effect (i.e. not lowering plaque pH below 5.7 during and up to 30 minutes after consumption) is not unique to Nutriose[®], but common to other “non-fermentable” carbohydrates (e.g. polyols, D-tagatose, isomaltulose, and polydextrose). A claim related to the “non-fermentable” carbohydrates xylitol, sorbitol, mannitol, maltitol, lactitol, isomalt, erythritol, D-tagatose, isomaltulose, sucralose and polydextrose and maintenance of tooth mineralisation by decreasing tooth demineralisation has

already been assessed with a favourable outcome (EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2011). The claim referred to the replacement of sugars in foods or drinks which reduce plaque pH below 5.7 by these food constituents (“non-fermentable” carbohydrates) or a combination, so that consumption of such foods or drinks does not lower plaque pH (as assessed by plaque pH telemetry) below 5.7 during and up to 30 minutes after consumption, and does not lead to dental erosion.

This Opinion applies to “non-fermentable” carbohydrates, which should replace “fermentable” carbohydrates in foods or beverages in order to obtain the claimed effect. The Panel notes that “non-fermentable” carbohydrates which have a neutral taste cannot substitute for the sweet taste of sugar.

The Panel considers that the food constituent, “non-fermentable” carbohydrates, which is the subject of the health claim, and the food constituent (i.e. “fermentable” carbohydrates) that “non-fermentable” carbohydrates should replace in foods or drinks are sufficiently characterised in relation to the claimed effect.

2. Relevance of the claimed effect to human health

The claimed effect is “maintenance of tooth mineralisation by decreasing tooth demineralisation”. The target population proposed by the applicant is the general population.

Demineralisation of tooth tissues can occur following acid production caused by the fermentation of carbohydrates by acid-producing bacteria in dental biofilms. The effect may be balanced by remineralisation when pH is neutralised and a state of calcium and phosphate supersaturation is achieved. If demineralisation is not balanced by remineralisation then net demineralisation of tooth tissues results which, if sustained, can lead to dental caries. Demineralisation of tooth tissues can also occur as a result of consumption of dietary acids in foods or beverages, and frequent consumption can lead to dental erosion. Dental caries and dental erosion are diseases with a high prevalence in the EU.

The Panel considers that maintaining tooth mineralisation by reducing tooth demineralisation resulting from acid production in plaque caused by the fermentation of carbohydrates is a beneficial physiological effect.

3. Scientific substantiation of the claimed effect

The evidence available from consensus opinions, reports from authoritative bodies and reviews shows that an increased risk of dental caries in children is associated with a high frequency (more than about four times daily) of intake of “cariogenic” sugars (mainly sucrose, glucose and fructose), rather than with the total amount of dietary sugars, and that frequent consumption of sweets, confectionery products and sugar-containing drinks is associated with a higher risk of caries (DoH, 1991; Moynihan and Petersen, 2004; IoM, 2005; Anderson et al., 2009; EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2010). Foods rich in starch, especially when the starch molecule is easily available to degradation by amylase, may also contribute (FDA, 1996; EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2010).

The evidence available from consensus opinions, reports from authoritative bodies and reviews also indicates that the decrease in pH in plaque as a consequence of metabolic acid production by saccharolytic bacteria when exposed to fermentable carbohydrates (i.e. sugars and starches) may promote demineralisation and prevent remineralisation of hydroxyapatite crystals. Tooth hydroxyapatite crystals are very resistant to dissolution at neutral pH, but their solubility drastically increases as pH drops. Typically, the critical pH for dental enamel is around 5.5. The Panel considers that foods lowering plaque pH, as determined *in vivo* or *in situ* by pH telemetry, below a conservative value of 5.7 by bacterial fermentation during and up to 30 minutes after consumption may promote demineralisation and prevent remineralisation of hydroxyapatite crystals (FDA, 1996). The Panel

notes that demineralisation of tooth tissues can also occur as a result of consumption of dietary acids in foods or beverages, and that frequent consumption can lead to dental erosion.

A claim on the sugar replacers xylitol, sorbitol, mannitol, maltitol, lactitol, isomalt, erythritol, D-tagatose, isomaltulose, sucralose and polydextrose and maintenance of tooth mineralisation by decreasing tooth demineralisation has already been assessed with a favourable outcome (EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2011).

The Panel notes that the sugar replacers mentioned above are “non-fermentable” carbohydrates as defined in this Opinion. The Panel also notes that the food constituents to be replaced in order to obtain the claimed effect, sugars, are “fermentable” carbohydrates as defined in this Opinion.

The Panel considers that the scientific substantiation and proposed conditions of use for the above-mentioned claim on sugar replacers also apply to “non-fermentable” carbohydrates, which should replace “fermentable carbohydrates” in foods and beverages in order to obtain the claimed effect.

The Panel concludes that a cause and effect relationship has been established between the consumption of foods/beverages containing “fermentable” carbohydrates at an exposure frequency of four or more times daily and an increased tooth demineralisation, and that the consumption of foods/beverages containing “non-fermentable” carbohydrates instead of “fermentable” carbohydrates may maintain tooth mineralisation by decreasing tooth demineralisation, provided that such foods/beverages do not lead to dental erosion.

The Panel could have reached this conclusion without the human studies claimed as proprietary by the applicant (Imfeld, 2002, 2010a, 2010b, unpublished).

4. Panel’s comments on the proposed wording

The Panel considers that the following wording reflects the scientific evidence: “Frequent consumption of “fermentable” carbohydrates contributes to tooth demineralisation. Consumption of foods/beverages containing “non-fermentable” carbohydrates instead of “fermentable” carbohydrates may help maintain tooth mineralisation by decreasing tooth demineralisation”.

5. Conditions and restrictions of use

The Panel considers that in order to bear the claim, “fermentable” carbohydrates should be replaced in foods or beverages (which reduce plaque pH below 5.7) by “non-fermentable” carbohydrates, so that consumption of such foods or beverages does not lower plaque pH below 5.7 during and up to 30 minutes after consumption, and does not lead to dental erosion.

The Panel notes that “non-fermentable” carbohydrates which have a neutral taste cannot substitute for the sweet taste of sugar.

CONCLUSIONS

On the basis of the data presented, the Panel concludes that:

- The food constituent, “non-fermentable” carbohydrates, which is the subject of the health claim, and the food constituent (i.e. “fermentable” carbohydrates) that “non-fermentable” carbohydrates should replace in foods or drinks are sufficiently characterised in relation to the claimed effect
- The claimed effect proposed by the applicant is “maintenance of tooth mineralisation by decreasing tooth demineralisation”. The target population proposed by the applicant is the

general population. Maintaining tooth mineralisation by reducing tooth demineralisation resulting from acid production in plaque caused by the fermentation of carbohydrates is a beneficial physiological effect.

- A cause and effect relationship has been established between the consumption of foods/beverages containing “fermentable” carbohydrates at an exposure frequency of four or more times daily and an increased tooth demineralisation; consumption of foods/beverages containing “non-fermentable” carbohydrates instead of “fermentable” carbohydrates may maintain tooth mineralisation by decreasing tooth demineralisation, provided that such foods/beverages do not lead to dental erosion.
- The following wording reflects the scientific evidence: “Frequent consumption of “fermentable” carbohydrates contributes to tooth demineralisation. Consumption of foods/beverages containing “non-fermentable” carbohydrates instead of “fermentable” carbohydrates may help maintain tooth mineralisation by decreasing tooth demineralisation”.
- In order to bear the claim, “fermentable” carbohydrates should be replaced in foods or beverages (which reduce plaque pH below 5.7) by “non-fermentable” carbohydrates, so that consumption of such foods or beverages does not lower plaque pH below 5.7 during and up to 30 minutes after consumption, and does not lead to dental erosion.

DOCUMENTATION PROVIDED TO EFSA

Health claim application on Nutriose® and maintenance of tooth mineralisation by decreasing tooth demineralisation pursuant to Article 13(5) of Regulation (EC) No 1924/2006 (Claim serial No: 0374_FR). January 2013. Submitted by Roquette Frères.

REFERENCES

- Anderson CA, Curzon ME, Van Loveren C, Tatsi C and Duggal MS, 2009. Sucrose and dental caries: a review of the evidence. *Obesity Reviews*, 10 Suppl 1, 41-54.
- DoH (Department of Health), 1991. Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. HMSO, London, United Kingdom.
- EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2010. Scientific Opinion on Dietary Reference Values for carbohydrates and dietary fibre. *EFSA Journal* 2010;8(3):1462, 40 pp. doi:10.2903/j.efsa.2010.1462
- EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2011. Scientific Opinion on the substantiation of health claims related to the sugar replacers xylitol, sorbitol, mannitol, maltitol, lactitol, isomalt, erythritol, D-tagatose, isomaltulose, sucralose and polydextrose and maintenance of tooth mineralisation by decreasing tooth demineralisation (ID 463, 464, 563, 618, 647, 1182, 1591, 2907, 2921, 4300), and reduction of post-prandial glycaemic responses (ID 617, 619, 669, 1590, 1762, 2903, 2908, 2920) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. *EFSA Journal* 2011;9(4):2076, 25 pp. doi:10.2903/j.efsa.2011.2076
- FDA (Food and Drug Administration), 1996. Food Labeling: Health Claims; Sugar Alcohols and Dental Caries. Federal Register 61 FR 43433.
- Imfeld T, 2002 (unpublished, claimed as proprietary by the applicant). Expertise des propriétés ménageant les dents d'un échantillon de produit (Nutriose® FB06) de la société Roquette Frères, 62080 Lestrem Cedex, France.
- Imfeld T, 2010a (unpublished, claimed as proprietary by the applicant). Expert's report on the telemetric evaluation of three products (Nutriose® FM06) provided by Roquette Frères, Lestrem, France, with regard to the products' qualification as being “safe for teeth”.

- Imfeld T, 2010b (unpublished, claimed as proprietary by the applicant). Expert’s report on the telemetric evaluation of a product (Nutriose®FB06) provided by Roquette Frères, Lestrem, France, with regard to the products’ qualification as being “safe for teeth”.
- IoM (Institute of Medicine), 2005. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty acids, Cholesterol, Protein, and Amino Acids. National Academies Press, Washington DC, United States of America.
- Moynihan PJ, 1998. Update on the nomenclature of carbohydrates and their dental effects. *Journal of Dentistry*, 26, 209-218.
- Moynihan P and Petersen PE, 2004. Diet, nutrition and the prevention of dental diseases. *Public Health Nutrition*, 7, 201-226.

SCIENTIFIC OPINION

Scientific Opinion on the substantiation of a health claim related to Nutriose®06 and a reduction of post-prandial glycaemic responses pursuant to Article 13(5) of Regulation (EC) No 1924/2006¹

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)^{2,3}

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Following an application from Roquette Italia S.P.A., submitted for authorisation of a health claim pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of Italy, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on the scientific substantiation of a health claim related to Nutriose®06 and a reduction of post-prandial glycaemic responses. Non-digestible carbohydrates, including resistant dextrin in Nutriose®06, are resistant to hydrolysis and absorption in the small intestine and therefore do not contribute to post-prandial glycaemia. This opinion applies to non-digestible carbohydrates (e.g. non-starch polysaccharides, resistant oligosaccharides and resistant starch), which should replace glycaemic carbohydrates in foods or beverages in order to obtain the claimed effect. The Panel considers that the food constituent, non-digestible carbohydrates, which is the subject of the health claim, and the food constituent (i.e. glycaemic carbohydrates) that non-digestible carbohydrates should replace in foods or beverages are both sufficiently characterised in relation to the claimed effect. The Panel considers that a reduction of post-prandial glycaemic responses might be a beneficial physiological effect. A claim on non-digestible carbohydrates and reduction of post-prandial glycaemic responses has already been assessed by the Panel with a favourable outcome. The previous evaluation, including the proposed wording and the conditions of use, also applies to this application. The Panel concludes that a cause and effect relationship has been established between the consumption of foods/beverages containing non-digestible carbohydrates and a reduction of post-prandial glycaemic responses as compared with foods/beverages containing glycaemic carbohydrates.

© European Food Safety Authority, 2014

KEY WORDS

resistant dextrin, Nutriose®06, non-digestible carbohydrates, post-prandial glycaemic responses, health claims

¹ On request from the Competent Authority of Italy following an application by Roquette Italia S.P.A., Question No EFSA-Q-2014-00073, adopted on 18 September 2014.

² Panel members: Carlo Agostoni, Roberto Berni Canani, Susan Fairweather-Tait, Marina Heinonen, Hannu Korhonen, Sébastien La Vieille, Rosangela Marchelli, Ambroise Martin, Androniki Naska, Monika Neuhäuser-Berthold, Grażyna Nowicka, Yolanda Sanz, Alfonso Siani, Anders Sjödin, Martin Stern, Sean (J.J.) Strain, Inge Tetens, Daniel Tomé, Dominique Turck and Hans Verhagen. Correspondence: nda@efsa.europa.eu

³ Acknowledgement: The Panel wishes to thank the members of the Working Group on Claims: Carlo Agostoni, Jean-Louis Bresson, Susan Fairweather-Tait, Marina Heinonen, Ambroise Martin, Hildegard Przyrembel, Yolanda Sanz, Alfonso Siani, Anders Sjödin, Sean (J.J.) Strain, Inge Tetens, Hendrik Van Loveren, Hans Verhagen and Peter Willatts for the preparatory work on this scientific opinion.

Suggested citation: EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2014. Scientific Opinion on the substantiation of a health claim related to Nutriose®06 and a reduction of post-prandial glycaemic responses pursuant to Article 13(5) of Regulation (EC) No 1924/2006. EFSA Journal 2014;12(10):3839, 9 pp. doi:10.2903/j.efsa.2014.3839

Available online: www.efsa.europa.eu/efsajournal

SUMMARY

Following an application from Roquette Italia S.P.A., submitted for authorisation of a health claim pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of Italy, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on the scientific substantiation of a health claim related to Nutriose®06 and a reduction of post-prandial glycaemic responses.

The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence. The application included a request for the protection of proprietary data.

The food that is the subject of the health claim is resistant dextrin in Nutriose®06, which should replace glycaemic carbohydrates in foods or beverages in order to obtain the claimed effect (i.e. reduction of post-prandial glycaemic responses). The Panel notes that the characteristic which is most relevant to the claimed effect is not unique to resistant dextrin but is common to other non-digestible carbohydrates (e.g. non-starch polysaccharides and resistant oligosaccharides) because, similar to resistant dextrin, non-digestible carbohydrates are resistant to hydrolysis and absorption in the small intestine and therefore do not contribute to post-prandial glycaemia. This opinion applies to non-digestible carbohydrates (e.g. non-starch polysaccharides, resistant oligosaccharides and resistant starch), which should replace glycaemic carbohydrates in foods or beverages in order to obtain the claimed effect. The Panel considers that the food constituent, non-digestible carbohydrates, which is the subject of the health claim, and the food constituent (i.e. glycaemic carbohydrates) that non-digestible carbohydrates should replace in foods or beverages are both sufficiently characterised in relation to the claimed effect.

The claimed effect proposed by the applicant relates to the reduction of post-prandial blood glucose responses. The target population proposed by the applicant is individuals wishing to reduce their post-prandial glycaemic responses. The Panel considers that a reduction of post-prandial glycaemic responses (as long as post-prandial insulinaemic responses are not disproportionately increased) might be a beneficial physiological effect.

The applicant submitted six unpublished human studies as being pertinent to the health claim.

A claim on non-digestible carbohydrates and reduction of post-prandial glycaemic responses has already been assessed by the Panel with a favourable outcome.

The Panel considers that the previous evaluation on the replacement of sugars with non-digestible carbohydrates applies to the replacement of all glycaemic carbohydrates with non-digestible carbohydrates, including the proposed wording and the conditions of use.

The Panel concludes that a cause and effect relationship has been established between the consumption of foods/beverages containing non-digestible carbohydrates and a reduction of post-prandial glycaemic responses as compared with foods/beverages containing glycaemic carbohydrates.

TABLE OF CONTENTS

Abstract	1
Summary	2
Table of contents	3
Background	4
Terms of reference	4
EFSA Disclaimer.....	4
Information provided by the applicant	6
Assessment	6
1. Characterisation of the food/constituent	6
2. Relevance of the claimed effect to human health.....	7
3. Scientific substantiation of the claimed effect	8
Conclusions	8
Documentation provided to EFSA	9
References	9

BACKGROUND

Regulation (EC) No 1924/2006⁴ harmonises the provisions that relate to nutrition and health claims, and establishes rules governing the Community authorisation of health claims made on foods. As a rule, health claims are prohibited unless they comply with the general and specific requirements of this Regulation, are authorised in accordance with this Regulation, and are included in the lists of authorised claims provided for in Articles 13 and 14 thereof. In particular, Article 13(5) of this Regulation lays down provisions for the addition of claims (other than those referring to the reduction of disease risk and to children's development and health) which are based on newly developed scientific evidence, or which include a request for the protection of proprietary data, to the Community list of permitted claims referred to in Article 13(3).

According to Article 18 of this Regulation, an application for inclusion in the Community list of permitted claims referred to in Article 13(3) shall be submitted by the applicant to the national competent authority of a Member State, which will make the application and any supplementary information supplied by the applicant available to the European Food Safety Authority (EFSA).

STEPS TAKEN BY EFSA

- The application was received on 03/02/2014.
- The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence. The application included a request for the protection of proprietary data.
- The scientific evaluation procedure started on 24/03/2014.
- On 07/05/2014, the Working Group on Claims of the NDA Panel agreed on a list of questions for the applicant to provide additional information to accompany the application, and the clock was stopped on 19/05/2014, in compliance with Article 18(3) of Regulation (EC) No 1924/2006.
- On 27/05/2014, EFSA received the requested information and the clock was restarted, in compliance with Article 18(3) of Regulation (EC) No 1924/2006.
- During its meeting on 18/09/2014, the NDA Panel, having evaluated the data submitted, adopted an opinion on the scientific substantiation of a health claim related to non-digestible carbohydrates and a reduction of post-prandial glycaemic responses.

TERMS OF REFERENCE

EFSA is requested to evaluate the scientific data submitted by the applicant in accordance with Article 16(3) of Regulation (EC) No 1924/2006. On the basis of that evaluation, EFSA will issue an opinion on the scientific substantiation of a health claim related to: non-digestible carbohydrates (including resistant dextrin in Nutriose®06) and a reduction of post-prandial glycaemic responses.

EFSA DISCLAIMER

The present opinion does not constitute, and cannot be construed as, an authorisation for the marketing of non-digestible carbohydrates, a positive assessment of their safety, nor a decision on whether non-digestible carbohydrates are, or are not, classified as a foodstuff. It should be noted that such an assessment is not foreseen in the framework of Regulation (EC) No 1924/2006.

⁴ Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. OJ L 404, 30.12.2006, p. 9–25.

It should also be highlighted that the scope, the proposed wording of the claim, and the conditions of use as proposed by the applicant may be subject to changes, pending the outcome of the authorisation procedure foreseen in Article 18(4) of Regulation (EC) No 1924/2006.

INFORMATION PROVIDED BY THE APPLICANT

Applicant's name and address: Roquette Italia S.P.A., Via Serravalle 26, 15063 Cassano Spinola, Alessandria, Italy.

The application includes a request for the protection of proprietary data for six unpublished studies (Donazzolo and Latreille-Barbier, 2000; GI labs, 2007; Thondre and Lightowler, 2012; Yuexin et al., 2012; Gendre, 2013; Salvi, 2013), in accordance with Article 21 of Regulation (EC) No 1924/2006.

Food/constituent as stated by the applicant

According to the applicant, the food that is the subject of the health claim is Nutriose[®]06, described as a resistant dextrin produced from wheat or maize starch.

Health relationship as claimed by the applicant

According to the applicant, the consumption of foods/drinks containing Nutriose[®]06 instead of high glycaemic carbohydrates leads to a reduced blood glucose rise. The applicant argues that this is owing to the fact that only 15 % of Nutriose[®]06 is hydrolysed in the upper digestive tract while 75 % are fermented in the large intestine and 10 % are excreted into the faeces. This low digestibility of Nutriose[®]06 is claimed to lead to a lower post-prandial glycaemia when replacing available carbohydrates in a meal, without any disproportionate increase in post-prandial insulinaemic response.

Wording of the health claim as proposed by the applicant

The applicant has proposed the following wording for the health claim: "Consumption of foods/drinks containing Nutriose[®]06 instead of high glycaemic carbohydrates induces a lower blood glucose rise after their consumption compared to high glycaemic carbohydrates-containing foods/drinks".

Specific conditions of use as proposed by the applicant

According to the applicant, high-glycaemic carbohydrates in foods or drinks should be replaced by Nutriose[®]06 so that foods or drinks contain reduced amounts of high-glycaemic carbohydrates, in accordance with the Annex of Regulation (EC) No 1924/2006. The proposed target population is individuals from the general population wishing to reduce their post-prandial glycaemic responses.

ASSESSMENT

1. Characterisation of the food/constituent

The food that is the subject of the health claim is Nutriose[®]06, which should replace "high glycaemic carbohydrates" in foods or beverages in order to obtain the claimed effect (i.e. reduction of post-prandial glycaemic responses).

Nutriose[®]06 is produced from wheat or maize starch by dry roasting under acidic conditions (i.e. dextrinisation). The ensuing dextrin is treated enzymatically and then purified by chromatographic partitioning in order to minimise the content of mono- and disaccharides. During the manufacturing process, linear and/or branched glycosidic bonds (i.e. α -1,2; α -1,3; β -1,2; β -1,3; β -1,4; β -1,6) are formed, which render dextrins resistant to digestion by salivary and pancreatic amylases. The final product contains about 85 % resistant dextrin with an average degree of polymerisation of 12-25, and

less than 0.5 % mono- and disaccharides. An overview of the manufacturing process, stability data and batch-to-batch variability were provided.

Following a request for clarification, the applicant indicated that the term “high-glycaemic carbohydrates” refers to “any carbohydrate rapidly digested and absorbed in the small intestine which induces a high increase in plasma glucose level, e.g. glucose, sucrose, maltose, glucose syrup, maltodextrin and high digestible starches”. The Panel notes that glycaemic carbohydrates include sugars, oligosaccharides and polysaccharides which can be digested and absorbed in the small intestine and contribute to post-prandial glycaemic responses (EFSA NDA Panel, 2010).

The Panel notes that the main characteristic of Nutriose®06 which contributes to the claimed effect is the non-digestibility of the resistant dextrin contained in Nutriose®06 in the small intestine, and that replacing digestible (glycaemic) carbohydrates by any non-digestible carbohydrate would contribute to the claimed effect. The applicant was requested to indicate which characteristics or properties of resistant dextrin in Nutriose®06 make it unique compared with other non-digestible carbohydrates (e.g. non-starch polysaccharides and resistant oligosaccharides) in relation to the claimed effect. In reply, the applicant stated that Nutriose®06 is manufactured through a “unique and patented manufacturing process during which transglycosylation reactions occur”. The applicant claims that the “unique arrangement” of the glycosidic bonds in Nutriose®06 is responsible for the low glycaemic and low insulinaemic response of Nutriose®06. The Panel notes that no evidence was provided to indicate that the resistant dextrin in the product was different from other non-digestible carbohydrates in relation to the claimed effect.

The Panel considers that in the context of this application the main characteristic of Nutriose®06 which contributes to the claimed effect (i.e. reduction of post-prandial glycaemic responses by replacing glycaemic carbohydrates in foods and beverages) is the non-digestibility of the resistant dextrin contained in Nutriose®06. The Panel also considers that this characteristic, which is relevant to the claimed effect, is not unique to resistant dextrin but is common to other non-digestible carbohydrates (e.g. non-starch polysaccharides and resistant oligosaccharides) because, similar to resistant dextrin, non-digestible carbohydrates are resistant to hydrolysis and absorption in the small intestine and therefore do not contribute to post-prandial glycaemia.

This opinion applies to non-digestible carbohydrates (e.g. non-starch polysaccharides, resistant oligosaccharides and resistant starch; EFSA NDA Panel, 2010), which should replace glycaemic carbohydrates in foods or beverages in order to obtain the claimed effect.

The Panel considers that the food constituent, non-digestible carbohydrates (including resistant dextrin in Nutriose®06), which is the subject of the health claim, and the food constituent (i.e. glycaemic carbohydrates) that non-digestible carbohydrates should replace in foods or beverages are both sufficiently characterised in relation to the claimed effect.

2. Relevance of the claimed effect to human health

The claimed effect proposed by the applicant relates to the reduction of post-prandial blood glucose responses. The target population proposed by the applicant is individuals wishing to reduce their post-prandial glycaemic responses.

The elevation of blood glucose concentrations after consumption of a food and/or meal, i.e. post-prandial glycaemia, is a normal physiological response which varies in magnitude and duration, and which may be influenced by the chemical and physical nature of the food or meal consumed, as well as by individual factors (Venn and Green, 2007). Decreasing post-prandial glycaemic responses may, for example, be beneficial to individuals with impaired glucose tolerance, as long as post-prandial insulinaemic responses are not disproportionately increased. Impaired glucose tolerance is common in the general population of adults.

The Panel considers that a reduction of post-prandial glycaemic responses (as long as post-prandial insulinaemic responses are not disproportionately increased) might be a beneficial physiological effect.

3. Scientific substantiation of the claimed effect

The applicant performed a literature search in Medline, using the search term “Nutriose”. Randomised controlled trials performed with Nutriose®06 in healthy subjects on post-prandial glycaemia were considered eligible. No such published studies were identified.

The applicant submitted six unpublished human studies (Donazzolo and Latreille-Barbier, 2000; GI labs, 2007; Thondre and Lightowler, 2012; Yuexin et al., 2012; Gendre, 2013; Salvi, 2013; all claimed as proprietary by the applicant) as being pertinent to the health claim. In these studies, the consumption of Nutriose®06 was shown to induce a significant reduction of post-prandial glycaemic and insulinaemic responses when compared with glucose.

A claim on non-digestible carbohydrates and reduction of post-prandial glycaemic responses has already been assessed by the Panel with a favourable outcome (EFSA NDA Panel, 2014). The Panel took into account that consumption of non-digestible carbohydrates results in reduced post-prandial blood glucose (and insulinaemic) responses compared with the consumption of sugars on a weight-by-weight basis owing to the non-digestibility in the small intestine and to a decrease in the amount of available carbohydrates, and that the consumption of foods/drinks in which non-digestible carbohydrates replaced sugars induced lower post-prandial glycaemic and insulinaemic responses than sugar-containing foods/drinks.

The Panel considers that the outcome, including the proposed wording and the conditions of use, of the previous evaluation on the replacement of sugars with non-digestible carbohydrates applies to the replacement of all glycaemic carbohydrates with non-digestible carbohydrates.

The Panel concludes that a cause and effect relationship has been established between the consumption of foods/beverages containing non-digestible carbohydrates and a reduction of post-prandial glycaemic responses as compared with foods/beverages containing glycaemic carbohydrates. The Panel notes that non-digestible carbohydrates have a neutral taste and cannot substitute for the sweet taste of sugars.

The Panel could have reached this conclusion without the human studies (Donazzolo and Latreille-Barbier, 2000; GI labs, 2007; Thondre and Lightowler, 2012; Yuexin et al., 2012; Gendre, 2013; Salvi, 2013) claimed as proprietary by the applicant.

CONCLUSIONS

On the basis of the data presented, the Panel concludes that:

- The food constituent, non-digestible carbohydrates (including resistant dextrin in Nutriose®06), which is the subject of the health claim, and the food constituent (i.e. glycaemic carbohydrates) that non-digestible carbohydrates should replace in foods or beverages, are both sufficiently characterised in relation to the claimed effect.
- The claimed effect proposed by the applicant relates to the reduction of post-prandial blood glucose responses. The target population proposed by the applicant is individuals wishing to reduce their post-prandial glycaemic responses. A reduction of post-prandial glycaemic responses might be a beneficial physiological effect.

- A cause and effect relationship has been established between the consumption of foods/beverages containing non-digestible carbohydrates and a reduction of post-prandial glycaemic responses as compared with foods/beverages containing glycaemic carbohydrates.

DOCUMENTATION PROVIDED TO EFSA

Health claim application on Nutriose®06 and reduction of post-prandial glycaemic responses pursuant to Article 13(5) of Regulation (EC) No 1924/2006 (Claim serial No: 0410_IT). January 2014. Submitted by Roquette Italia S.P.A.

REFERENCES

- Donazzolo Y and Latreille-Barbier M, 2000 (unpublished, claimed as proprietary by the applicant). Evaluation des index glycémiques et insulinémiques du LAB2454 chez des volontaires sains. Clinical study report, No OP35199VS.ROQ, 131 pp.
- EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition, and Allergies), 2010. Scientific Opinion on Dietary Reference Values for carbohydrates and dietary fibre. EFSA Journal 2010;8(3):1462, 77 pp. doi:10.2903/j.efsa.2010.1462
- EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2014. Scientific Opinion on the substantiation of a health claim related to non-digestible carbohydrates and reduction of post-prandial glycaemic responses pursuant to Article 13(5) of Regulation (EC) No 1924/2006. EFSA Journal 2014;12(1):3513, 13 pp. doi:10.2903/j.efsa.2014.3513
- Gendre D, 2013 (unpublished, claimed as proprietary by the applicant). Détermination de la réponse glycémique post-prandiale au NUTRIOSE® chez le volontaire sain. Clinical study report, No PEC12127, 109 pp.
- GI labs, 2007 (unpublished, claimed as proprietary by the applicant). Determination of the glucose and insulin responses to NUTRIOSE® and reduced sugar corn syrup products. Clinical study report, No GIL-6030, 13 pp.
- Salvi A, 2013 (unpublished, claimed as proprietary by the applicant). To evaluate the glycemic and insulinaemic responses of a test product (NUTRIOSE® FB06) in healthy volunteers. Clinical study report, No CL/012/0712 STU, 36 pp.
- Thondre S and Lightowler H, 2012 (unpublished, claimed as proprietary by the applicant). Glycaemic and insulinaemic response for NUTRIOSE FB06 and anhydrous dextrose. Clinical study report, 8 pp.
- Venn BJ and Green TJ, 2007. Glycemic index and glycemic load: measurement issues and their effect on diet-disease relationships. European Journal of Clinical Nutrition, 61 (Suppl. 1), S122-131.
- Yuexin Y, Zhu W, Xuesong X and Jing L, 2012 (unpublished, claimed as proprietary by the applicant). Chinese human study - NUTRIOSE®FB06 – Glycaemic and insulinaemic responses. Clinical study report, No CPS 12-216, 30 pp.

Safety Assessment of Polysaccharide Gums as Used in Cosmetics

Status: Final Report
Release Date: October 22, 2015
Panel Date: September 21-22, 2015

The 2015 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is Lillian J. Gill, D.P.A. This report was prepared by Wilbur Johnson, Jr., M.S., Senior Scientific Analyst and Bart Heldreth, Ph.D., Chemist.

ABSTRACT

The Cosmetic Ingredient Review (CIR) Expert Panel (the Panel) reviewed the safety of 106 ingredients, which function as viscosity increasing agents in cosmetic products. The Panel reviewed relevant animal and human data on these ingredients. The Panel concluded that most of the polysaccharide gums are safe in the present practices of use and concentration in cosmetics, as described in this safety assessment, but that the available data are insufficient to make a determination that hydrolyzed carrageenan is safe under the intended conditions of use in cosmetics. The Panel was concerned about the presence of alkylating and other agents that are used to modify polysaccharide gums in cosmetics. Industry should use good manufacturing practices to limit impurities.

INTRODUCTION

The safety of 106 polysaccharide gums (see Tables 1 and 2) as used in cosmetics is reviewed in this safety assessment. The polysaccharide gums are each naturally derived materials that comprise polysaccharides obtained from plants or algae. Based on the different chemical structures that are associated with polysaccharide gums, these ingredients can be subdivided into categories such as modified, unmodified, linear, branched, and cyclic. Regardless of how they are structured, all of the “moieties” that comprise the molecular structures of these ingredients are polymers composed of monosaccharides.

Although these ingredients could be categorized in multiple ways, all of these ingredients fall into two predominate categories, modified and unmodified. The ingredients in the Modified subgroup have been further subdivided into Linear, Branched, Cyclic, and Unknown Structural Configuration. The ingredients in the Unmodified subgroup have been subdivided into Linear Polysaccharides and Their Salts, Branched - Unmodified, Cyclic, and Unknown Structural Configuration.

Based on chemical similarities, relevant data on the following are included for use in evaluating the safety of ingredients in this review: wheat bran extract (contains ~ 80% arabinoxylan oligopeptides) - for use in the safety assessment of arabinoxylan (branched - unmodified subgroup); pectin-derived acidic oligosaccharides (mixture of linear oligomers and small polymers of galacturonic acid) - for safety assessment of pectin (branched - unmodified subgroup), which consists chiefly of partially methoxylated polygalacturonic acids; and carboxymethyl inulin - for safety assessment of sodium carboxymethyl inulin (branched - modified subgroup). Many of the polysaccharide gums reviewed in this safety assessment function as viscosity increasing agents in cosmetic products.¹ Other functions are listed in Table 2.

As a group, polysaccharide gums comprise polymers of simple saccharide monomers. Their substantial molecular sizes suggest that skin penetration of these ingredients would be unlikely. Thus, these ingredients are unlikely to have significant systemic accessibility and any major decomposition products are likely to be simple saccharides.

In addition, the Panel has issued “safe as used” conclusions for the following cosmetic ingredients which are structurally similar to some of the ingredients reviewed in this safety assessment: galactomannans,² microbial polysaccharide gums,³ astragalus gummifer gum,^{4,5} aloe barbadensis leaf polysaccharides,⁶ oryza sativa (rice) starch,⁷ zea mays (corn) starch,⁸ acacia senegal gum,⁹ glyceryl alginate,¹⁰ hyaluronic acid,¹¹ and triticum vulgare (wheat) starch.^{12,13}

CHEMISTRY

Definition and Structure

Polysaccharide nomenclature follows the general principles of established organic and carbohydrate nomenclature. Polysaccharide (glycan) is the name given to a macromolecule consisting of a large number of monosaccharide (glycose) residues joined to each other by glycosidic linkages (Figure 1). The term poly(glycose) is not a synonym for polysaccharide (glycan), because it refers to macromolecules composed of glycose residues joined to each other by non-glycosidic linkages. Polysaccharides may be linear, branched, or cyclic. Definitions, structures, and functions of the polysaccharide gums reviewed in this safety assessment, as used in cosmetics and defined in the *International Cosmetic Ingredients Dictionary and Handbook*, are presented in Tables 1 and 2.¹

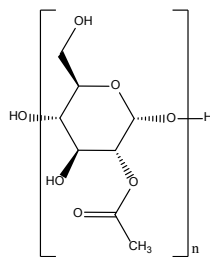


Figure 1. Starch Acetate – an example of a polysaccharide gum

The polysaccharide gums are each naturally derived materials that comprise polysaccharides obtained from plants or algae. Their substantial molecular sizes suggest that skin penetration of these ingredients would be unlikely. While, for the sake of clarity and organization, these ingredients can be subdivided into categories such as linear, branched, cyclic, modified, and unmodified, these moieties represent a family of structurally similar polymeric materials, composed of simple saccharide monomers. So, in intended cosmetic application, these ingredients are unlikely to have significant systemic accessibility and any major decomposition products are likely to be simple saccharides, albeit chemically modified ones in some instances (*vide supra*).

Physical and Chemical Properties

Physical and chemical properties of polysaccharide gums are presented in Table 3. These gums have high molecular weights, and many are insoluble in water.

Method of Manufacture

Methods of manufacture of polysaccharide gums are presented in Table 3. The manufacturing processes for hydrolyzed furcellaran and starch hydroxypropyltrimonium chloride are presented in the following sections.

Linear – Modified

Hydrolyzed Furcellaran

The manufacturing process for hydrolyzed furcellaran is presented in Figure 2 below.

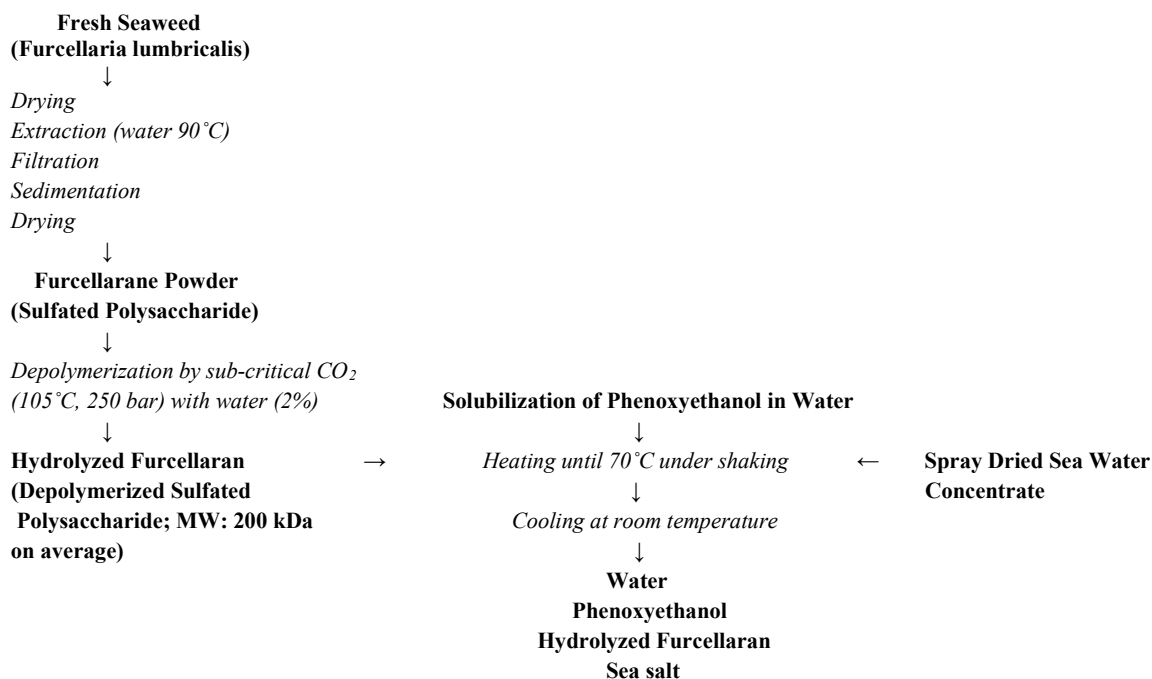


Figure 2. Manufacturing Process for Hydrolyzed Furcellaran.¹⁴

Branched – Modified

Starch Hydroxypropyltrimonium Chloride

The manufacturing process for starch hydroxypropyltrimonium chloride is presented in Figure 3 below.

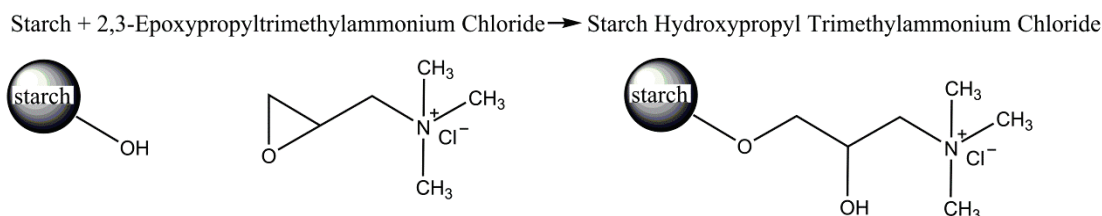


Figure 3. Reaction to form cationic starch ether.¹⁵

Composition/Impurities

Composition and impurities data on polysaccharide gums are presented in Table 4. Composition/properties data on two hydrolyzed starch products are presented in Table 5.

USE

Cosmetic

Many of the ingredients reviewed in this safety assessment function as viscosity increasing agents in cosmetic products, and the complete list of polysaccharide gum functions in cosmetic products is presented in Table 2.¹ According to information supplied to the Food and Drug Administration (FDA) by industry as part of the Voluntary Cosmetic Registration Program (VCRP), and the results from a survey of ingredient use concentrations conducted by the Personal Care Products Council (Council) in 2013, 58 of these polysaccharide gums are being used in cosmetic products and maltodextrin has the highest reported use frequency.^{16,17,18,19}

The Council survey data also indicate that polysaccharide gums are being used in rinse-off cosmetic products at maximum ingredient use concentrations up to 50% (i.e., for algin in paste masks and mud packs), and in leave-on cosmetic products at maximum ingredient use concentrations up to 45.7% (i.e., for corn starch modified in tonics, dressings, and other hair grooming aids).^{16,18} Frequency of use/use concentration data for polysaccharide gums are summarized in Table 6.

Cosmetic products containing polysaccharide gums may be applied to the skin and hair or, incidentally, may come in contact with the eyes (maximum ingredient use concentration in these products = 30%) and mucous membranes (maximum ingredient use concentration in these products = 32%). Products containing these ingredients may be applied as frequently as several times per day and may come in contact with the skin or hair for variable periods following application. Daily or occasional use may extend over many years.

Polysaccharide gums are used at concentrations up to 9.5% (avena sativa (oat) starch) in cosmetic products that are sprayed, which also includes use in a pump hair spray at a maximum concentration of 0.45% (corn starch modified), and at concentrations up to 45.7% (corn starch modified) in cosmetic products that possibly are sprayed. Ingredient use in underarm aerosol deodorant sprays is being reported at maximum use concentrations ranging from 0.001% (algin) to 2.5% (cyclodextrin). Hydroxypropyl cyclodextrin is being used in underarm pump deodorant sprays at a maximum use concentration of 0.34%. Additionally, polysaccharide gums are used in powders at concentrations up to 33% (tapioca starch). Because polysaccharide gums are used in products that are sprayed, they could possibly be inhaled. In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters >10 µm, with propellant sprays yielding a greater fraction of droplets/particles below 10 µm, compared with pump sprays.^{20,21,22,23} Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.^{20,21} There is some evidence indicating that deodorant spray products can release substantially larger fractions of particulates having aero-

dynamic equivalent diameters in the range considered to be respirable.²¹ However, the information is not sufficient to determine whether significantly greater lung exposures result from the use of deodorant sprays, compared to other cosmetic sprays.

Non-cosmetic

According to the FDA, the following polysaccharide gums are approved direct food additives affirmed as generally recognized as safe (GRAS):^{24,25} agar, alginic acid, ammonium alginate, amylose (i.e., high-amylose corn starch is GRAS), calcium alginate, pectin, potassium alginate, dextrin, maltodextrin, solanum tuberosum (potato) starch, solanum tuberosum (potato) starch, starch acetate, tapioca starch, hydroxypropyl starch, propylene glycol alginate, carrageenan, ghatti gum, and sterculia urens gum.

Linear Polysaccharides and Their Salts

Algin

The viscosity of blood substitutes is among the important determinants in restoring microcirculation.²⁶ Sodium alginate (algin) is frequently mentioned as a viscosity modifier in the development of blood substitutes.

Alginates

Alginate dressings are among the types of absorbent dressings that are used to treat exuding wounds.²⁷

Carrageenan

κ-Carrageenan (thickening agent) stabilizes milk proteins and is widely used in dairy products.²⁸

At the June 2014 meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the Committee concluded that the use of carrageenan in infant formula or formula for special medical purposes at concentrations up to 1000 mg/L is not of concern.²⁹ Furthermore, the Committee recognized that there is variability in medical conditions among infants requiring formulas for special medical purposes that contain the higher levels of carrageenan, and noted that these infants would normally be under medical supervision. A summary of the discussion on which the Committee's conclusion is based is summarized in the Repeated Dose Toxicity-Oral section of this report.

Inulin

Inulin is a prebiotic, meaning a non-digestible food ingredient that selectively stimulates the growth and/or activity of one or several bacterial species in the colon.³⁰

Branched - Unmodified

Ghatti Gum

Ghatti gum (thickening agent) is used to stabilize table syrup emulsions, as a glaze in candy products, and as a component of chewing gum, cough drops, and candy lozenges.²⁸

Sterculia Urens Gum

Sterculia urens gum has the following uses in food: formulation aid, stabilizer and thickener, and emulsifier and emulsifier salt.³¹ World Health Organization (WHO) reports affirming the safety of karaya gum as a food additive are available.^{32,33}

Cyclic

Cyclodextrin

Cyclodextrins have been used to solubilize drugs in aqueous vehicles as guest-host complexes.³⁴

TOXICOKINETICS

Non-Human

Linear Polysaccharides and Their Salts

Carrageenan

Carrageenan is not degraded or absorbed in the gastrointestinal tracts of rodents, dogs, and non-human primates.³⁵

Branched - Unmodified

Sterculia Urens Gum

A toxicokinetic study on sterculia urens gum was performed using 2 groups of 4 male Sprague-Dawley rats of the CD strain. One group was fed a pelleted diet containing 5% sterculia urens gum for 24 h, and the control group was fed a similar laboratory pelleted diet without the gum. Urine and feces were collected and weighed after 24 h, 48 h, and 72 h. The polysaccharide of sterculia urens gum is composed essentially of rhamnose, galactose and galacturonic acid. Fecal polysaccharide was calculated as sterculia urens gum polysaccharide after correction for background levels of rhamnose, galactose, and galacturonic acid in the control feces. The quantity and monosaccharide composition of the fecal polysaccharide were compared with the dose and original composition of the gum polysaccharide. Aggregated polysaccharide estimated over the 72-h collection period ranged from 81% to 108%, with a mean value of 95% of that consumed. Thus, 95% of the gum ingested was excreted in the feces.³⁶

Cyclic

Cyclodextrin

The absorption of orally administered ¹⁴C-β-cyclodextrin, in methylcellulose solution, was studied using 4 Wistar R x Long Evans F₁ male rats.³⁷ Two rats received an oral dose of 36.7 mg/kg, and the other 2 rats received 36.9 mg/kg. The average dose volume was 1.5 ml. The maximum radioactivity of the blood derived from ¹⁴C-β-cyclodextrin occurred between the 4th and 11th hour after exposure, and the maximum radioactivity in different experiments ranged from 5% to 17% of the total administered radioactivity. Radioactivity excreted in the urine ranged from 4.2% to 4.8% of the total radioactivity administered. No specific accumulation of ¹⁴C-β-cyclodextrin in organs was found after dosing. The large intestine contained 10% to 15% of the ¹⁴C-β-cyclodextrin radioactivity at 24 h post-dosing.

In another experiment, a female CFY rat received an oral dose of 313 mg/kg ¹⁴C-β-cyclodextrin (homogenized in dextran solution, volume = 2.5 ml). In the 8th hour after dosing, no more than 3 to 50 ppm β-cyclodextrin was detectable in the blood. In a third experiment, a female CFY rat was dosed orally with 36.1 mg/kg ¹⁴C-β-cyclodextrin (homogenized in 1 ml dextran solution), and another rat was dosed orally with 313.5 mg/kg ¹⁴C-β-cyclodextrin (homogenized in 2.5 ml dextran solution). Three female CFY rats also received an oral dose of 1.88 mg/kg chromatographically purified ¹⁴C-β-cyclodextrin (homogenized in 1.5 ml dextran solution). The radioactivity peak was detected in the exhaled air between the 4th to 6th or the 6th to 8th hour, depending on the dose. The total radioactivity exhaled by ¹⁴C-β-cyclodextrin-treated rats in 24 h represented 55% to 64% of the administered ¹⁴C-β-cyclodextrin. The authors suggested, based on the results of this study, that the rate-determining step in β-cyclodextrin absorption is the enzymatic hydrolysis of β-cyclodextrin to yield linear dextrans, which are rapidly hydrolyzed to maltose and glucose.³⁷

Human

Branched - Unmodified

Starch Acetate

The pharmacokinetics of starch acetate (acetyl starch) and hydroxyethyl starch was studied using 2 groups of 16 surgical patients (18 to 70 years old).³⁸ Patients in one group were initially infused intravenously (i.v.) with 15 mL/kg of a 6% acetyl starch solution, and then up to a maximal dosing volume of 1,000 mL/kg, over a 30-minute period. The other group was infused with a 6% hydroxyethyl starch solution (same dosing volume) according to the same procedure. When compared to hydroxyethyl starch, rapid and nearly complete enzymatic degradation to acetic acid and glucose (and to products that can be excreted renally) was reported for acetyl starch.

Sterculia Urens Gum

Five male volunteers were involved in a study in which 24-h urine samples were collected prior to, and following, the ingestion of 10 g karaya gum for 15 days.³⁹ Total gum intake was 10-fold greater than the approved average daily intake (ADI) of 0-12.5 mg/kg body weight. The detection limit for rhamnose in the urine was 0.2 µg; however, rhamnose was not detected in any of the urine specimens. The authors noted that if 1% of the rhamnose in 10 g karaya gum appeared in the 24-h urine specimens, it would have been detected. Furthermore, the results of this study confirmed that dietary gum karaya is neither digested nor degraded by enteric bacteria, and is not absorbed to any significant extent in the digestive tract.

Tapioca Starch

Ten men (29 to 41 years old) participated in an oral exposure study.⁴⁰ Blood was collected after a 12-h fast. Tapioca starch (30 g) containing 0.1 g aspartame was dissolved in 150 L of water, and the solution or dispersion remained for 3 minutes in boiling water. Subjects then drank the solution 1 to 2 min later. Three tolerance tests were performed, using a crossover design, over three days. Tapioca starch produced a large, rapid increase in plasma glucose concentration, which peaked in 30 minutes and then decreased toward the basal value.

Percutaneous Absorption

Cyclic - Modified

Hydroxypropyl Cyclodextrin

The percutaneous absorption of 2% ¹⁴C-2-hydroxypropyl-β-cyclodextrin *in vivo* was studied using 3 to 5 female hairless mice.⁴¹ The test material (100 µL on occlusive patch) was applied to dorsal skin (2 cm²) for 24 h. Radioactivity in the patches, in the stratum corneum (collected by tape stripping), and in the epidermis and cutis of the skin (obtained by peeling off the treated portion) was measured using a scintillation counter. The percutaneous absorption of ¹⁴C-2-hydroxypropyl-β-cyclodextrin through intact skin was extremely low, i.e., ~ 0.02% of the amount applied to the skin. The absorption rate of ¹⁴C-2-hydroxypropyl-β-cyclodextrin through skin from which the stratum corneum had been removed by tape stripping was approximately 24% of the amount applied to the skin. The latter finding suggests that the stratum corneum may act as a barrier to the percutaneous absorption of ¹⁴C-2-hydroxypropyl-β-cyclodextrin. Thus, the results of this study clearly demonstrate that 2-hydroxypropyl-β-cyclodextrin has low permeability through hairless mouse skin.

TOXICOLOGICAL STUDIES

A toxicity profile of β-cyclodextrin (a cyclic polysaccharide gum) is available from the WHO.⁴² The toxicity profile of cyclodextrins can differ depending on the route of administration. For example, β-cyclodextrin administered orally induces limited toxicity.^{43,44} In both rats and dogs, β-cyclodextrin is considered to be non-toxic at a daily dose less than 600 mg/kg body weight or at 3% or less in the diet.⁴⁵ However, if β-cyclodextrin is administered at higher doses in animals via a subcutaneous (s.c.) route, it will cause a decrease in body weight gain, a decrease in liver weight, and nephrotoxicity, with an increase in kidney weight, proximal tubular nephrosis and cellular vacuolation.^{45,46} In another study (rats), s.c. administration of β-cyclodextrin (≥ 450 mg/kg) induced similar changes in kidney proximal tubules.⁴⁷ Acute and repeated dose toxicity studies on polysaccharide gums (according to type of exposure) are summarized in Tables 7 and Table 8, respectively. The following acute toxicity studies (according to type of exposure) on polysaccharide gums are summarized in Table 7: inhalation, oral, dermal,

intravenous, intrapleural, and transbronchial. Oral and dermal repeated dose toxicity studies on polysaccharide gums are summarized in Table 8.

Cytotoxicity

Linear Polysaccharides and Their Salts

Calcium Alginate

In a cytotoxicity assay, calcium alginate fibers were introduced into human embryonic kidney cells and human fibroblasts.⁴⁸ A total of nine experimental groups were prepared according to the following weights of calcium alginate fibers: 0.005, 0.01, 0.02, 0.03, 0.04, 0.05, 0.08, 0.10, and 0.15 g. Next, 1-cm lengths of fibers were cut and sterilized with UV irradiation prior to their addition to the cells. The cells were in their exponential growth phase, and were incubated for 48 h. Calcium alginate fibers were not cytotoxic.

Allergenicity/Immune System Effects

Non-Human

Linear Polysaccharides and Their Salts

Polianthes Tuberosa Polysaccharide

The potential for a modulatory effect on the murine self-defense system by an acidic polysaccharide (ANK-102) produced by *Polianthes tuberosa* cells in liquid culture was examined.⁴⁹ The pretreatment (intraperitoneal [i.p.] injection) of C3H/HeN mice with ANK-102 (2 mg in 0.2 ml solution) deteriorated murine survival against lethal infection with *Listeria monocytogenes*, an intracellular gram positive bacterium eliminated mainly by macrophages through the T-cell mediated immune response. Pretreatment with ANK-102 resulted in the accumulation of Mac 1 and Mac 2 positive cells in the peritoneal cavity of the infected animals and the reduction of Thy 1.2 expression on the surface of the thymocytes. ANK-102 was classified as an immunosuppressive polysaccharide.

Potassium Carrageenan

Male Sprague-Dawley rats (8 animals, 7 weeks old) were injected i.p. with potassium carrageenan (50 mg in 5 ml PBS).⁵⁰ The control group received a single injection of PBS (0.5 ml). At 3 weeks post-injection, serum levels of IgM, IgG and slow α_1 - and slow α_2 -globulins were measured using quantitative radial immunodiffusion (IgG) or immunoelectrophoresis (IgM and slow α -globulins). There was a significant elevation in levels of IgM and slow α_1 globulin that was maximal on day 4; levels returned to normal by day 14. Slow α_2 -globulin was detectable within 24 h, reached a peak at day 2, and, in most animals, was no longer measurable by day 14. Levels of IgG were not affected by potassium carrageenan injection.

Branched - Unmodified

Sterculia Urens Gum (a.k.a. Karaya Gum)

The allergenicity of karaya gum was studied in adult male and female guinea pigs (number not stated).⁵¹ Karaya gum (1 g/kg) was dissolved in normal saline to make a 3% solution, which was injected i.p. The gum was also administered orally (1 g/animal daily) for 3 months, or mixed with food (single feeding of 5 g/animal). Egg albumen served as the control in each experiment. Animals that received single i.p. injections or single oral doses were killed at intervals within a range of 4 to 12 weeks after the attempted sensitization. Animals dosed orally daily for 3 months were killed either on the day after the last dose or after an interval of 6 weeks after the last dose. Isolated pieces of small intestine from treated males and females, seminal vesicles from males, and the uterus of females were suspended in an organ bath and exposed to karaya gum or egg albumen for 10 minutes. The organs of

animals exposed *in vivo* to karaya gum where challenged first with egg albumen and, later, with karaya gum, and *vice versa*. Study results indicated that allergic sensitivity did not develop in guinea pigs dosed orally (single or repeated doses) or i.p. Injection of albumen resulted in marked allergic sensitization.

An animal model was used to investigate the immunogenicity of karaya gum (*Sterculia* spp.).⁵² Groups of [(C57BL/6J x DBA/2)F₁] (BDF₁) mice were intradermally immunized with the gum in Freund's complete adjuvant. Serum antibody levels were measured using an enzyme-linked immunosorbent assay (ELISA), and delayed hypersensitivity responses assayed by a footpad swelling test. Karaya gum elicited systemic immune responses after immunization. Further processing reduced immunogenicity, although there was no evidence that systemic immunity to complex polysaccharide antigen responses could be completely abolished by processing or purification. Karaya gum caused considerable footpad swelling when injected intradermally.

Human

Branched - Modified

Propylene Glycol Alginate

Following a 7-day control period, 5 male volunteers consumed propylene glycol alginate at a dose of 175 mg/kg body weight for 7 days.⁵³ This regimen was followed by dosing with 200 mg/kg body weight for an additional 16 days. No allergic responses were reported by, nor observed in, any of the volunteers.

In Vitro

Linear Polysaccharides and Their Salts

Potassium Alginate

The acute tissue reactions to potassium alginate, locally applied to a microvascular bed, were studied using the vital microscopic hamster cheek-pouch model and correlative histology.⁵⁴ This experimental model permitted the study of microvascular permeability, blood flow, vessel diameters and leucocyte adhesion to vessel walls intravitaly, and leucocyte migration and mast cell degranulation histologically. Deionized water alone and potassium alginate with flavor and color mixed in saline was found to cause severe microvascular alterations, while potassium alginate, without flavor and color, mixed in saline and applied to the microvasculature resulted in a minor inflammatory reaction

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Reproductive and developmental toxicity data on polysaccharide gums are summarized in Table 9. Except for a dose-dependent increase (40-600 mg/kg) in the incidence of missing skeletal sternebrae in rabbits dosed orally with *kappa/lambda*-carrageenan, the results for polysaccharide gums in reproductive and developmental toxicity studies were essentially negative.

GENOTOXICITY

Genotoxicity data (bacterial and mammalian) on polysaccharide gums are summarized in Table 10. In bacterial assays, the following were not genotoxic either with or without metabolic activation: arabinoxylan, carboxymethyl inulin, carrageenan, ghatti gum, glucomannan, and pectin-derived acidic oligosaccharides. In mammalian assays with and without metabolic activation, wheat bran extract, carboxymethyl inulin, carrageenan, ghatti gum, and glucomannan were not genotoxic. However, results for pectin-derived acidic oligosaccharides in mammalian assays were either equivocal or it was classified as clastogenic, only at highly cytotoxic concentrations. *Sterculia urens* gum was not genotoxic in cytogenetic assays (*in vitro* and *in vivo*) or in the *in vivo* dominant lethal gene test.

CARCINOGENICITY

Studies relating to the carcinogenicity of polysaccharide gums are summarized in Table 11. Agar (50,000 ppm in diet) was not carcinogenic in rats, and up to 25% sodium alginate in the diet was not carcinogenic in mice. Results relating to the carcinogenic potential of carrageenan were mixed. Carrageenan (25% in the diet) was not carcinogenic in mice, but 15% carrageenan in the diet enhanced the colon tumor incidence in azoxymethane (AOM)- and N-nitrosomethylurea (NMU)-treated rats. In the aberrant crypt focus (ACF) assay, 10% carrageenan in the diet did not initiate colon tumors, 0.25% carrageenan reduced the number of ACF, and 2.5% carrageenan promoted the growth of ACF in rats. In another study, carrageenan (up to 5% in the diet) did not possess promoting activity for colorectal carcinogenesis in rats. It should also be noted that 5% carrageenan in the diet increased colonic cell proliferation in rats, but that it was concluded that this response was probably adaptive, and would not contribute to the increased risk of colon neoplasia in rats. There was no evidence of carcinogenicity in mice fed 55% starch acetate or in rats fed 5% cyclodextrin in the diet. Pectin (2.5% in diet) caused mucosal hyperplasia of the small intestine of rats. Degraded carrageenan, which may or may not be similar to the cosmetic ingredient hydrolyzed carrageenan, caused colon cancer in rats at dietary concentrations of 5% and 10%, but not 1%, in rats. Degraded carrageenan (also known as poligeenan) results from a manufacturing process of seaweed that involves intentional extensive acid hydrolysis, resulting in sulfated galactose polymers with an average molecular weight of approximately 15,000 Da.³⁵

Inulin (15 g in basal diet) inhibited the growth of 2 tumor cell lines that were implanted in mice, and the dietary intake of 4.8% arabinoxylan reduced the occurrence of preneoplastic lesions in rats. Glucomannan (10% in the diet) inhibited the development of spontaneous liver tumors in mice.

IRRITATION AND SENSITIZATION

Dermal Irritation and Sensitization

Skin irritation and sensitization studies on polysaccharide gums are summarized in Table 12. The results of animal and human tests indicate that these gums can be mild skin irritants, but are non-sensitizers.

Phototoxicity

Branched - Modified

Sodium Hydrolyzed Potato Starch Dodecenylsuccinate

The phototoxicity of a sodium hydrolyzed potato starch dodecenylsuccinate was evaluated using the *in vitro* neutral red uptake phototoxicity assay.⁵⁵ The trade name material (in Hanks' balanced salt solution) was evaluated at concentrations ranging from 68.1 to 1,000 µg/ml in BALB/3T3 clone A31 mouse embryo fibroblast cultures. Chlorpromazine served as the positive control. Following incubation, cultures were irradiated for 50 minutes with 1.7 mW/cm² UVA to achieve an irradiated dose of 5 J/m². A positive result was defined as a photo-irritant factor (PIF) > 5. The PIF was defined as the EC₅₀ without solar simulated light (SSL)/ EC₅₀ with SSL. The test material was not considered to have phototoxicity potential (PIF = 0.8). A PIF of 27.9 was reported for the chlorpromazine positive control.

Clinical Trial

Linear Polysaccharides and Their Salts

Calcium Alginate

Fourteen patients (7 males) with spina bifida were treated for pressure sores. Each patient had calcium alginate dressings applied for 4 to 6 weeks.⁵⁶ The mean number of dressings removed per week was 3.5 ± 2.1. Good tolerance to treatment was reported for each patient. It was also noted that no severe side effects were recorded during the trial.

Case Reports

Linear Polysaccharides and Their Salts

Calcium Alginate

A 50-year-old woman was referred for treatment after the discovery of adenoid cystic carcinoma in an excised left submandibular gland.⁵⁷ Treatment involved clearing the left submandibular fossa, and selective neck dissections. After removal of the clot (submandibular hematoma), a calcium alginate fiber pack was left in place to control the bleeding. After an extended period, the pack was reported to have stimulated a foreign body reaction which, on a computed tomogram, mimicked a recurrence of the tumor.

Alginate

A 52-year-old general practitioner injected 0.1 ml of an alginate solution into the deep dermis of her left arm.⁵⁸ Ten days later, she observed a small pink nodule at the injection site; a bluish papule was observed at 3 months post-injection. A biopsy was performed 2 months after injection. At histopathological examination, a granulomatous reaction involving the deep dermis and the subcutaneous fat was observed. The papule regressed, having resolved completely at 5 months post-injection.

Four of 10 patients injected with an aesthetic injectable resorbable filler consisting of purified alginate (extracted from crusted brown algae), into tear troughs and/or dorsa of the hands, developed severe granulomatous reactions within months after injections.⁵⁹ The 40% incidence of this disfiguring effect was considered high.

Sodium Carrageenan

Within minutes of receiving a barium enema solution that contained sodium carrageenan, a 26-year-old female had an anaphylactic reaction associated with the following signs/symptoms:⁶⁰ abdominal cramps, mild generalized pruritus, generalized urticaria, hypotension, transient loss of consciousness, chest tightness, wheezing, and cyanosis. A skin prick test for a component of the barium enema solution, 0.4% weight/volume sodium carrageenan, were positive (i.e., an 8 mm wheal diameter with surrounding flare). This is the only component of the barium enema solution that yielded a positive reaction.

Ocular Irritation

Non-Human

Linear Polysaccharides and Their Salts

Algin

The ocular irritation potential of algin (2%) was studied in 3 experiments using rabbits (number not stated).⁶¹ Instillation of the test substance was followed by scoring after 1 h, 24 h, 2 days, 3 days, 4 days, and 7 days. Corneal opacity and ulceration or granulation were evaluated. Ocular irritation was graded on a scale of 0 to 110, and an ocular irritation index (OII) was calculated. It was noted that a compound does not provoke any significant injury to the mucous membrane of the eye when no opacity of the cornea occurs and when the ocular irritation index is less than 15. OII values of 3.00, 9.17, and 5.50 were reported in the 3 experiments, respectively. Pathological lesions of the ocular mucosa were not observed.

Carrageenan

Food grade *iota*-carrageenan (one subtype of carrageenan with a specific number and position of sulfate groups on the repeating galactose units) was not irritating to unrinsed eyes of rabbits and was minimally irritating to rinsed eyes.⁶²

Branched – Modified

Calcium Starch Isododecenylsuccinate and Corn Starch Modified

A material described as structurally similar to sodium hydrolyzed potato starch dodecenylsuccinate and corn starch modified was evaluated for ocular irritation potential in a study involving 6 New Zealand White rabbits.^{63,64,65} The OECD 405 test protocol was used. The powder (0.1 ml) was placed in one eye of each animal. Iritis was observed in 2 rabbits, and reactions had cleared by day 1. Conjunctival irritation was observed in 6 rabbits, and reactions had cleared by day 3. There was no evidence of corneal opacity or abnormal systemic signs during the observation period. The test material was classified as a minimal ocular irritant.

Corn Starch Modified

Corn starch modified, dry powder form, was placed in one eye of each of 6 New Zealand White rabbits (5 males, 1 female).⁶⁶ Iritis was observed in 1 of 6 rabbits, and the reaction had cleared by 24 h post-administration. Mild conjunctival irritation was observed in all 6 rabbits, and reactions had cleared by 48 h post-administration. There was no evidence of corneal opacity or abnormal physical signs in any of the animals tested. The test substance was classified as minimally irritating to the eye.

Dextrin Myristate

The ocular irritation potential of dextrin myristate was studied using 6 New Zealand white rabbits. The test concentration and protocol were not stated. Ocular irritation was not observed.⁶⁷

Dextrin Palmitate

In an ocular irritation study involving 3 New Zealand white rabbits per test substance, dextrin palmitate (concentration and test protocol not stated) did not cause reactions in the cornea or iris. Slight conjunctival redness was observed in one rabbit at 1 h post-instillation, but had resolved after 24 h.^{68,69}

Potato Starch Modified

A 16.8% aqueous suspension of potato starch modified was evaluated in an ocular irritation study involving 3 rabbits (strain not stated), according to the OECD 405 test guideline. Conjunctival irritation/edema was observed in the 3 rabbits, and all reactions had cleared in 2 rabbits by 24 h post-instillation. In the remaining rabbit, slight swelling of the conjunctivae remained at 24 h, and the reaction had cleared by 48 h post-instillation. It was concluded that the potato starch modified suspension was slightly irritating to the eyes of rabbits.

The ocular irritation potential of potato starch modified (28-1808) was evaluated according to the OECD 405 protocol using 3 New Zealand White rabbits.⁷⁰ An 18.5% solids solution of the test substance (0.1 ml) was instilled into one eye of each animal, and reactions were scored for up to 72 h post-instillation. Abnormal physical signs were not observed during the observation period. Conjunctival irritation was observed in all animals, having cleared by 48 h. Neither corneal opacity nor iritis was observed during the study. Potato starch modified (28-1808) was classified as a minimal ocular irritant.

Stearoyl Inulin

The ocular irritation potential of stearoyl inulin (test concentrations and protocol not stated) was evaluated in two tests, each using 6 Japanese white rabbits. The test substance was classified as practically non-irritating.^{71,72}

In Vitro

Linear - Modified

Hydrolyzed Furcellaran

The ocular irritation potential of a trade name mixture containing 1.35% furcellaran powder and 1% phenoxyethanol was evaluated in a cytotoxicity assay involving cultured fibroblasts (source not stated). The method of diffusion on agarose gel was used. The product (pure) was applied to cultures during a 24-h period, and was classified as slightly toxic. This finding was interpreted as almost non-irritating to slightly irritating to the eyes.⁷³ The ocular irritation potential of another trade name mixture containing 1.35% furcellaran powder, 0.1% potassium sorbate, and 0.05% citric acid was evaluated according to the same procedure, and the same results were reported.⁷³

Maltodextrin

The ocular irritation potential of maltodextrin was evaluated using the *in vitro* bovine corneal opacity and permeability assay.⁷⁴ In this assay, plastic cassettes mimicking eye structure are used as holders for excised corneas. The posterior chamber was filled with cell support media, and the anterior chamber was filled with an eye gel containing 2.45% maltodextrin. After a 10-minute period, opacity was measured by passing visible light from an opacitometer through the cornea and on to the surface of a light sensor. It was noted that a clear cornea unchanged by the test substance would allow light to pass through and be detected by the sensor. Opaque corneas would produce light scattering (Tyndall effect) and reduced detection that is proportional to the degree of ocular damage. Also, following exposure, fluorescein was added to the anterior chamber of the cassette. The amount of dye passing through the cornea and into the posterior chamber is a measure of corneal permeability, and an increase in corneal permeability is indicative of corneal damage. Based on the results of this study, the eye gel was classified as a non-irritant. The positive control, 5% benzalkonium chloride, was classified as a severe irritant.

In addition, the EPI-Ocular® skin model assay was used to evaluate the ocular irritation potential of an eye gel containing 2.45% maltodextrin.⁷⁵ In this assay, the degree of ocular irritation is based on the amount of cytotoxicity observed in tissues exposed to the test substance. Cytotoxicity is measured using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye. The end point established in this assay is the time required for the test substance to reduce tissue viability by 50% (ET₅₀). An ET₅₀ > 4 h (non-irritant) was reported for the eye gel. The positive control, Triton X-100, was classified as a mild irritant (ET₅₀ = 28.8 minutes).

Branched – Modified

Hydroxypropyltrimonium Hydrolyzed Corn Starch

The ocular irritation potential of hydroxypropyltrimonium hydrolyzed corn starch was evaluated using the hen's egg test – utilizing the chorioallantoic membrane (HET-CAM).⁷⁶ Fertile White leghorn eggs were used. The chorioallantoic membrane (CAM) of the chick embryo responds to injury with a complete inflammatory reaction that is comparable to that induced in the rabbit ocular irritation test. The test substance (0.3 ml) was administered to the CAM at concentrations of 5%, 10%, and 15%. Results indicated that hydroxypropyltrimonium hydrolyzed corn starch would have practically no irritation potential *in vivo*. It was noted that the CAM results at 5%, 10%, and 15% are equivalent to Draize test results for the test substance at concentrations of 10%, 20%, and 30%.

Mucous Membrane Irritation and Sensitization

Non-Human

Branched - Unmodified

Glucomannan

Konjac flour was evaluated in the following study, but the composition of konjac flour is not stated. However, according to one source, every 100 g of konjac flour contains the following:⁷⁷ glucomannan (79.37 mg), protein (1.64 g), fat (0.004 g), phosphorus (57 mg), iron (4.06 mg), zinc (123 mg), manganese (0.2 mg), chromium (0.25 mg), and copper (0.08 mg). Prior to initiation of the study, a sensory irritation study on konjac flour (primary polysaccharide component is glucomannan) was performed using ND4 Swiss Webster mice (number not stated).⁷⁸ Sensory irritation was evaluated by monitoring the decrease in respiratory rate during 30 minutes of exposure to

konjac flour. The concentration of konjac flour that caused a 50% decrease in the respiratory rate (RD_{50}) was 110 mg/m^3 .

A study was performed to investigate whether exposure to food grade konjac flour could produce respiratory hypersensitivity.⁷⁸ The composition of the sample tested was in agreement with *Food Chemical Codex* specifications of <8% protein, >75% carbohydrate, and <5% ash. Groups of male Hartley guinea pigs were randomly assigned to the following 4 groups (whole-body exposure in chambers): negative control (4 animals, air-exposed), positive control (4 animals, trimellitic anhydride [TMA] exposure), and konjac flour exposure group (8 animals). Test animals were exposed to konjac flour on days 1-5 of the study (42 minutes/induction exposure), and challenged (35 minutes/challenge exposure) on days 19, 26, and 40. The mean ($\pm$ S.D.) konjac flour concentration during induction exposure was $111 \pm 8.3 mg/m^3$, and the mean exposure concentration during the challenge phase ranged from 50 to 68 mg/m^3 . The days of exposure (induction and challenge) for positive control animals exposed to TMA aerosol were identical to those for the test group. The target exposure concentration of TMA was 94 mg/m^3 for induction and challenge. Negative control animals were exposed to room air on days 1-5, but were challenged with konjac flour (target concentration = 114 mg/m^3) only on day 40 to avoid the possibility of repeated challenges resulting in sensitization.

The criteria used to define respiratory tract sensitization (increase in respiratory rate of 36% and change in respiratory waveform) were achieved in 25% of the animals during each challenge in the konjac flour exposure group. Additionally, a few animals responded with slightly lower increases in respiratory frequency and a change in waveform that were suggestive of a slight pulmonary hypersensitivity response.⁷⁸ According to a more recent publication, the purified antigen from konjac flour is named Ag40D-2 (acidic protein; ~ 24,000 daltons), suggesting that the respiratory sensitizer in konjac flour is actually a protein, rather than glucomannan.⁷⁹

Cyclic - Modified

Methyl Cyclodextrin

The acute histological effects of methylated β -cyclodextrin on the epithelium of the nasal cavity has been investigated in rats using light microscopy.⁸⁰ After a single nasal administration of 2% randomly methylated β -cyclodextrin, only minor changes were observed in the appearance of the cilia and the apical cell membranes, and small amounts of mucus were excreted into the nasal cavity. These effects were similar to those noted for control animals dosed with physiological saline (0.9% NaCl). Using confocal laser scanning microscopy, no changes in nasal epithelial cell morphology were observed after a single intranasal administration of 2% randomly methylated β -cyclodextrin, whereas 1 % sodium taurodihydrofusidate resulted in swelling of the cells and substantial mucus extrusion.

Human

Branched - Unmodified

Glucomannan

The inhalation of konjac dust in factories producing konnyaku, a popular food in Japan made from konjac tubers, has been reported to produce allergic bronchial asthma (known as konnyaku asthma) in sensitized individuals.⁸¹ Furthermore, bronchial asthma that was likely triggered by the inhalation of Maiko powder has been associated with residents near a konjac milling plant in Japan.⁷⁹ Konjac root is dried and ground into powder in the process of manufacturing the food known as konjac. Maiko is a fine konjac root powder that is blown by air pressure to obtain konjac powder for commercial use.

EPIDEMIOLOGY

Linear Polysaccharides and Their Salts

Carrageenan, Agar, and Alginate

An epidemiology study was performed to examine the hypothesis that the increasing incidence of mammary carcinoma in the United States in the twentieth century may be related to the consumption of carrageenan and possibly other water-soluble polymers.⁸² A time-trend analysis using age-adjusted incidence data and consumption data from established sources was used to test this hypothesis. Statistical analysis, using Pearson and Spearman correlation coefficients, was performed to identify associations between water-soluble polymer consumption and cancer incidence. Lag periods of 10, 15, 20, 25, 30, and 35 years were introduced to consider a latent effect between intake and the occurrence of breast cancer.

At least 4 values for consumption and corresponding incidence were required for inclusion in the correlation analysis. Consumption data on the polysaccharide gums studied were reported as pounds/person/year. These water-soluble polymer utilization data, obtained from several libraries throughout the United States, were predominantly from published data compiled as research for the food industry. For carrageenan, 80% of total consumption was identified as food consumption, and the remainder was attributed to products such as toothpaste, deodorants, room deodorizers, etc. Food consumption data on other gums were as follows: sterculia urens gum (< 10%), agar (50%), alginates (60%), and pectin (80 to 95%). Incidence data for breast cancer were obtained from published sources and were presented as the age-adjusted incidence data per 100,000 population using the 1970 census data.

The following positive correlations between gum consumption and the incidence of mammary carcinoma were found. For carrageenan, positive correlations (statistically significant) were found at 25 years ($r = 0.88$; $P = 0.048$) and 30 years ($r = 0.96$; $P = 0.042$). The Spearman correlation coefficient for carrageenan at 30 years was also statistically significant ($r = 1.0$; $P < 0.0001$). Statistically significant positive correlations were also reported for alginate (at 30-year lag period) and agar (at 10- and 25-year lag periods). The Spearman correlation coefficient was significant for pectin at 30 years. Sterculia urens gum did not demonstrate any statistically significant correlations. This analysis demonstrated that polysaccharide gum consumption correlated positively with increased incidence of breast carcinoma.

Branched - Unmodified

Pectin and Sterculia Urens Gum

Epidemiology data on pectin and sterculia urens gum are included in the preceding study on carrageenan, agar and alginate.⁸²

MISCELLANEOUS STUDIES

Endocrine Function and Vitamin D Absorption

Branched - Unmodified

Glucomannan

A double-blind trial on the efficacy of glucomannan in the treatment of pediatric obesity was performed.⁸³ The study involved 60 children under the age of 15 (mean age: 11.2 years; mean overweight: 46%). Thirty children received 1 g of glucomannan twice daily for two months, and the other 30 children received a placebo according to the same schedule. Clinical side effects were evaluated in both groups by measuring indicators of intestinal absorption, lipid metabolism, and thyroid and adrenocortical function. When the 2 groups were compared, there were no significant differences in intestinal absorption, thyroid or adrenocortical function, or clinical symptoms. However differences in lipid metabolism were significant. The treated group had decreased α -lipoprotein and increased pre- β -lipoprotein and triglyceride. The authors suggested that the metabolic alteration observed may have been due to a primary decrease in α -lipoprotein, most likely because of inadequate water intake. It was noted that these study results question the efficacy of glucomannan in the treatment of childhood obesity.

Antifungal Activity

Linear Polysaccharides and Their Salts

Calcium Alginate

The antifungal properties of calcium alginate fiber were studied using *Candida albicans*.⁴⁸ Fungal inhibitory rates were measured using the plate-count method, following the shake-flask test. Additionally, an inhibition-zone test and observation by scanning electron microscopy were performed. The inhibitory rate of calcium alginate fibers was 49.1%, and was classified as weak when compared to zinc alginate (92.2% inhibitory rate). The inhibitory rate was calculated using the following equation: Inhibitory rate = $[(A - B)/A] \times 100\%$. A was defined as the number of fungal colony on blank control plates. B was defined as the number of fungal colony on test plates.

Muscle Inflammation

Linear Polysaccharides and Their Salts

Carrageenan

Local muscle inflammation was induced by injecting carrageenan (10 mg/kg) into the right tibialis anterior muscle in 22 healthy ARC mice (6 weeks old).⁸⁴ The contralateral muscle was injected with sterile isotonic saline, and the muscles were removed after 24 h for measurement of contractile function and cytokine concentration. Carrageenan significantly reduced maximum specific force, decreased the maximum rate of force development, altered the force-frequency relationship, and increased intramuscular levels of pro-inflammatory cytokines and chemokines. These results indicate that injected carrageenan directly affects contractile function and causes skeletal muscle weakness.

Anti-inflammatory/Antioxidant Activity

Linear Polysaccharides and Their Salts

Alginic Acid

Alginic acid, isolated from brown algae (*Sargassum wightii*), was evaluated in a study involving groups of 6 arthritic adult male Sprague-Dawley rats.⁸⁵ The oral dosing of alginic acid (100 mg/kg) in arthritic rats reduced paw edema and the activities of enzymes such as cyclooxygenase, lipoxigenase and myeloperoxidase. Reduction in the level of C-reactive protein, ceruloplasmin, and rheumatoid factor were also observed in arthritic rats treated with alginic acid. Additionally, reduced lipid peroxidation and enhanced activities of antioxidant enzymes were reported, which suggest the antioxidant potential of the compound. Histopathological analysis indicated that alginic acid treatment reduced paw edema and inflammatory infiltration in arthritic rats. Overall, study results suggest that alginic acid isolated from *Sargassum wightii* exhibits potent anti-inflammatory and antioxidant activity.

SUMMARY

The polysaccharide gums are naturally derived materials that comprise polysaccharides obtained from plants or algae. As a group, they comprise polymers of simple saccharide monomers. Based on the different chemical structures that are associated with polysaccharide gums, these ingredients can be subdivided into categories such as modified, unmodified, linear, branched, and cyclic. Many of the polysaccharide gums reviewed in this safety assessment function as viscosity increasing agents in cosmetic products. According to information supplied to the FDA by industry as part of the VCRP and results from a Council survey of ingredient use concentrations, 59 polysaccharide gums are being used in cosmetic products.

The Council survey data also indicate that polysaccharide gums are being used in cosmetics at maximum ingredient use concentrations up to 50% (i.e., for algin in paste masks and mud packs). Polysaccharide gums are used at concentrations up to 9.5% (avena sativa (oat) starch) in cosmetic products that are sprayed, which also includes use in a pump hair spray at a maximum concentration of 0.45% (corn starch modified), and at concentrations up to 45.7% (corn starch modified) in cosmetic products that possibly are sprayed. Additionally, polysaccharide gums are used in cosmetic products (powders) at concentrations up to 33% (tapioca starch). Because polysaccharide gums are used in products that are sprayed, they could possibly be inhaled.

Maltodextrin, the most frequently used cosmetic ingredient reviewed in this safety assessment, is prepared as a white powder or concentrated solution by partial hydrolysis of corn starch, potato starch, or rice starch. It is an approved direct food additive affirmed as GRAS by the FDA. The following other polysaccharide gums reviewed in this safety assessment have also been classified as GRAS direct food additives: agar, alginic acid, ammonium alginate, amylose (i.e., high amylose corn starch is GRAS), calcium alginate, pectin, potassium alginate, dextrin, solanum tuberosum (potato) starch, starch acetate, tapioca starch, hydroxypropyl starch, propylene glycol alginate, ghatti gum, and sterculia urens gum.

In 2014, the JECFA concluded that the use of carrageenan in infant formula or formula for special medical purposes at concentrations up to 1,000 mg/L is not of concern.

Data on native carrageenans extracted from different types of algae indicate that different types of carrageenan have reasonable stability to heating at 75°C down to pH 4, and that the rate of depolymerization increases dramatically as the pH decreases from 4 to 3. These data indicate the susceptibility of carrageenan to acid hydrolysis under certain conditions.

The results of a percutaneous absorption study involving hairless mouse skin indicate that 2-hydroxypropyl- β -cyclodextrin had extremely low permeability, approximately 0.02% of the amount applied to the skin.

In studies involving rats, there was no specific accumulation of orally administered cyclodextrin in organs, and it was rapidly hydrolyzed to maltose and glucose. In another study, 95% of ingested sterculia urens gum was excreted in the feces of rats. Carrageenan was not degraded or absorbed from the gastrointestinal tract of rodents, dogs, and non-human primates, and rapid and nearly complete enzymatic degradation of starch acetate was reported. Dietary sterculia urens gum was neither digested nor degraded by enteric bacteria in humans, which is similar to what was observed in rats. In a human oral feeding study on tapioca starch, a rapid increase in plasma glucose was observed after dosing.

An $LC_{50} > 0.0015$ mg/l was reported for glucomannan in an acute inhalation toxicity study involving rats. The transbronchial injection of 0.75% carrageenan (in physiological saline) induced pneumonia in rabbits.

Acute oral dosing of rats with sterculia urens gum at a dose of 10 g/kg body weight did not cause death, and the same was true for rats dosed with 5,000 mg/kg potato starch modified, 5,000 mg/kg calcium starch isododecenylsuccinate (considered structurally similar to sodium hydrolyzed potato starch dodecenylsuccinate and corn-starch modified), 2,000 mg/kg corn starch modified, 2,000 mg/kg dextrin palmitate, 2,000 mg/kg dextrin myristate, or 2,000 mg/kg stearoyl inulin. Acute oral LD_{50} values of $> 2,800$ mg/kg body weight (mice) and $> 5,000$ mg/kg body weight (rats) have been reported for glucomannan.

In acute dermal toxicity studies on corn starch modified, potato starch modified, dextrin myristate, and dextrin palmitate, an LD_{50} of $> 2,000$ mg/kg (rats) was reported. The same results were reported for glucomannan in an acute dermal toxicity study involving rabbits.

Repeated dose oral toxicity studies on the following were performed: algin (25% in diet, mice) starch acetate (55% in diet, mice), arabinoxylan (~ 80% arabinoxylan oligopeptides in wheat bran extract [extract test concentrations up to 7.5% in diet], rats), inulin (7.5% in diet, rats), carboxymethyl inulin (31.1% aqueous at doses up to 1,000 mg/kg/day, rats), carrageenan (up to 5% in diet [rats]; up to 25% in diet [mice]; up to 500 mg/kg/day [monkeys]), cyclodextrin (up to 50,000 ppm in diet [rats]; up to 20% in diet [dogs]), ghatti gum (up to 5% in diet, rats), glucomannan (up to 8% in diet, rats), pectin (up to 10% pectin-derived acid oligosaccharides in diet, rats),

solanum tuberosum (potato) starch (up to 10% in diet, rats), and sterculia urens gum (5 g/kg/day, rats; 7% in diet, rats). Sodium alginate was nephrotoxic in mice, but results for starch acetate were of little, if any, toxicological significance. The NOAEL for wheat bran extract in rats was 4.4 g/kg/day, the highest dose administered; there were no remarkable findings in control rats dosed with inulin. There were no toxicologically significant findings in rats dosed with carboxymethyl inulin, and the same was true for ghatti gum. The liver and kidney were identified as target organs for toxicity in rats dosed with β -cyclodextrin, but there was no evidence of systemic toxicity in dogs. There were no treatment-related effects in dogs dosed with γ -cyclodextrin. Treatment-related histopathological changes in the urinary bladder were observed in rats fed pectin-derived acidic oligosaccharides in the diet. No adverse effects were observed in rats dosed repeatedly with sterculia urens gum. Transient fatty degeneration, with focal necrosis of the liver was observed in rats fed glucomannan in the diet.

Repeated oral feeding of humans with propylene glycol alginate (up to 200 mg/kg/day) or sterculia urens gum (10.5 g in diet/day) did not cause toxicity.

Systemic toxicity was not observed in guinea pigs that received repeated dermal applications of 31.1% aqueous carboxymethyl inulin, or in rats dosed dermally (2 g/kg body weight/day) with potato starch modified.

There were no changes in cell morphology of the nasal epithelium of rats after intranasal administration of methyl cyclodextrin.

Pathological lesions of the ocular mucosa were not observed after 2% algin was instilled into the eyes of rabbits. Carrageenan was non-irritating to the unrinsed eyes of rabbits, but was minimally irritating to rinsed eyes. Ocular irritation was not observed in rabbits tested with dextrin myristate, dextrin palmitate, or stearyl inulin. An eye gel containing 2.45% maltodextrin was classified as a non-irritant in the *in vitro* bovine corneal opacity and permeability assay, and in the *in vitro* EPI-Ocular® assay. Corn starch modified and calcium starch isododecenylsuccinate (considered structurally similar to sodium hydrolyzed potato starch dodecenylsuccinate and corn-starch modified) were minimally irritating to the eyes of rabbits. Potato starch modified and a 16.8% aqueous suspension of potato starch modified were slightly irritating to the eyes of rabbits. Hydroxypropyltrimonium hydrolyzed corn starch had practically no irritation potential at concentrations of 5%, 10%, and 15% in the *in vitro* HET-CAM ocular irritation assay. Mixtures containing 1.35% hydrolyzed furcellaran were classified as slightly toxic in a cytotoxicity assay involving cultured fibroblasts, and this finding was classified as almost non-irritating to slightly irritating to the eyes.

In a primary skin irritation study, results were negative for 2% algin in rabbits. In a cumulative skin irritation study involving rabbits, the results observed at macroscopic or microscopic examination indicated that 2% algin did not induce a severe reaction. Potato starch modified (10% solids aqueous solution) caused minimal to slight acanthosis in rabbits, and a 50% slurry of calcium starch isododecenylsuccinate (considered structurally similar to sodium hydrolyzed potato starch dodecenylsuccinate and corn-starch modified) was mildly irritating to the skin of rabbits. At a dose of 2,000 mg/kg in an acute dermal toxicity study, corn starch modified (30% solids in distilled water) was classified as a mild skin irritant in rabbits.

Skin irritation was not observed in albino guinea pigs patch-tested with 100% carboxymethyl inulin. Erythema and edema were observed in an acute dermal toxicity study involving rats dosed with 2 g/kg potato starch modified; all reactions cleared by 72 h. Neither erythema nor edema was observed in rats that received repeated dermal applications of the same dose of potato starch modified. Dextrin palmitate or dextrin myristate did not cause skin irritation in rabbits or skin sensitization in guinea pigs evaluated in the maximization test. A trade name mixture containing 1.35% hydrolyzed furcellaran was classified as non-irritating to the skin of human subjects. A trade name mixture containing 0.6% hydrolyzed furcellaran was classified as non-irritating and non-sensitizing when applied to the skin of human subjects.

In the guinea pig maximization test, corn starch modified (20% solution) and 31.1% aqueous carboxymethyl inulin did not induce sensitization. In the Buehler test for skin sensitization, potato starch modified (18.5% aqueous suspension) caused faint erythema during induction, but there was no evidence of sensitization in animals tested. Also, in the Buehler test, a paste of 50% calcium starch isododecenylsuccinate (considered structurally similar to sodium hydrolyzed potato starch dodecenylsuccinate and corn-starch modified) was not a

sensitizer in guinea pigs. ι -Carrageenan and konjac flour (glucomannan is primary polysaccharide component; the antigen is an acidic protein [AG40D-2]) were also non-sensitizing to the skin of guinea pigs.

Corn starch modified (7.5%) did not induce cumulative skin irritation in 26 subjects or skin sensitization in 113 subjects tested. A 50% w/v slurry or 50% solids slurry of calcium starch isododecenylsuccinate (considered structurally similar to sodium hydrolyzed potato starch dodecenylsuccinate and corn-starch modified) was classified as a probable mild irritant in a 21 day cumulative skin irritation study involving 23 human subjects.

Algae exopolysaccharides (1%) did not cause skin irritation or sensitization in an HRIPT involving 50 subjects. An eye gel containing 2.45% maltodextrin did not induce allergic contact dermatitis in an HRIPT involving 103 subjects. Results were negative for skin irritation and allergic contact dermatitis in 12 male subjects patch-tested with 20% aqueous sodium alginate. Negative results for skin sensitization were also reported for 227 subjects in a human RIPT on a cleanser containing 10 wt% sodium hydrolyzed potato starch dodecenylsuccinate. Neither skin irritation nor sensitization was observed in the following HRIPT's: 54 subjects tested with a rinse-off facial product containing 42.69% dextrin, 51 subjects tested with a leave-on facial product containing 0.3% dextrin myristate, and 47 subjects tested with hydroxypropyltrimonium hydrolyzed corn starch (15%).

Allergenicity was not associated with the oral dosing of human subjects with propylene glycol alginate, and dermal application of a calcium alginate dressing to patients did not cause any side effects that were classified as severe.

Sodium hydrolyzed potato starch dodecenylsuccinate was evaluated for phototoxicity at concentrations ranging from 68.1 to 1,000 $\mu\text{g/ml}$ in the *in vitro* neutral red uptake phototoxicity assay (BALB/3T3 clone A31 mouse embryo fibroblast cultures). The test material was not considered to have phototoxicity potential.

The concentration of konjac flour that caused a 50% decrease in respiratory rate (RD_{50}) in mice in a sensory irritation evaluation was 110 mg/m^3 . In a subsequent study, the criteria used to define respiratory tract sensitization (increase in respiratory rate of 36% and change in respiratory waveform) were achieved in 25% of the 8 guinea pigs challenged with konjac flour (mean exposure concentration range = 50 to 68 mg/m^3). The inhalation of konjac dust in factories producing konnyaku, a popular food in Japan made from konjac tubers, has been reported to produce allergic bronchial asthma in sensitized individuals.

In studies evaluating effects on the immune system, an acidic polysaccharide produced by *Polianthes tuberosa* cells was classified as an immunosuppressive polysaccharide. The injection (i.p.) of potassium carrageenan into rats resulted in significant elevation of serum IgM, but not IgG.

In pregnant mice that received doses of kappa/lambda-carrageenan (from *C. crispus*, sodium or calcium salt) at oral doses up to 900 mg/kg/day during gestation, there was a dose-dependent decrease in the number of live pups and in pup weight. Skeletal maturation was also retarded. In another study in which pregnant mice received oral doses of the same test substance (sodium or calcium salt) at doses up to 600 mg/kg/day during gestation, there was a dose-dependent increase in the incidence of missing skeletal sternebrae. However, feeding with the test substance (calcium salt) at dietary concentrations up to 5% prior to mating in a three-generation feeding study, no specific external, skeletal, or soft-tissue anomaly could be correlated with dosage. In a study in which calcium carrageenan was fed at dietary concentrations up to 1.8% prior to mating, during breeding, and throughout gestation, lactation, and post-weaning, there were no differences between test and negative control groups regarding length of gestation, litter size, or sex distribution.

The oral dosing of pregnant hamsters with doses of kappa/lambda-carrageenan (from *C. crispus*, sodium or calcium salt) up to 600 mg/kg/day during gestation resulted in some evidence of a dose-dependent delay in skeletal maturation. In a similar study in which hamsters received oral doses of the test substance (sodium or calcium salt) up to 200 mg/kg/day during gestation, there were no dose-related teratogenic or fetotoxic effects. When pregnant rabbits were dosed orally with the test substance (sodium or calcium salt) at doses up to 600 mg/kg/day during gestation, the numbers of skeletal or soft tissue abnormalities did not differ from those of controls.

Neither reproductive nor developmental toxicity was observed in rat dietary feeding studies on cyclodextrin (up to 20%), and pectin-derived acidic oligosaccharides (10%). Sterculia urens gum was not teratogenic when

administered in a corn oil suspension to rats (doses up to 900 mg/kg/day) rabbits (doses up to 635 mg/kg/day) or mice (doses up to 170 mg/kg/day) during gestation. Cyclodextrin also did not cause reproductive or developmental toxicity in rabbits when administered at dietary concentrations up to 20%, and the same was true when pregnant cats were fed 2% glucomannan in the diet during gestation.

In bacterial assays, the following were not genotoxic either with or without metabolic activation: arabinoxylan, carboxymethyl inulin, carrageenan, corn starch modified, ghatti gum, glucomannan, a trade name mixture containing 0.6% hydrolyzed furcellaran, pectin-derived acidic oligosaccharides, calcium starch isododecenylsuccinate (considered structurally similar to sodium hydrolyzed potato starch dodecenylsuccinate and corn-starch modified), and a sodium hydrolyzed potato starch dodecenylsuccinate tradename material. In mammalian assays with and without metabolic activation, wheat bran extract, carboxymethyl inulin, carrageenan, ghatti gum, and glucomannan were not genotoxic. However, results for pectin-derived acidic oligosaccharides in mammalian assays were either equivocal or it was classified as clastogenic. *Sterculia urens* gum was not genotoxic in cytogenetic assays (*in vitro* and *in vivo*) or in the *in vivo* dominant lethal gene test.

Agar, isolated from *Pterocladia*, was not carcinogenic in F344 rats or B6C3F₁ mice that received concentrations of 25,000 ppm or 50,000 ppm in the diet. Neither algin (25% in diet) nor starch acetate (55% in diet) was found to be carcinogenic in an oral feeding study involving mice. When fed in the diet to rats, carrageenan (up to 25% in diet), and cyclodextrin (up to 675 mg/kg/day), also were not carcinogenic. Carrageenan (up to 5% in diet) was not carcinogenic when fed to hamsters. In a co-carcinogenicity study, carrageenan (15% in the diet) enhanced the incidence of colon tumors in female Fischer 344 rats injected with azoxymethane or *N*-nitrosomethylurea.

Colorectal tumors were found in Sprague-Dawley rats fed 5% or 10% degraded carrageenan, but not 1% degraded carrageenan, in the diet for up to 24 months. Colorectal tumors were also observed in Sprague-Dawley rats that received 5% degraded carrageenan in drinking water for 15 months, and in Sprague-Dawley rats dosed with 1 g/kg or 5 g/kg degraded carrageenan by gastric intubation for 15 months. Fischer 344 rats that received 10% degraded carrageenan in the diet for up to 9 months also had colorectal tumors.

The feeding of rats with an inulin-enriched diet (10% in diet) resulted in the promotion of adenoma growth. Mucosal hyperplasia in the small intestine was observed in rats fed 2.5% pectin in the diet. In another feeding study, 5% methoxylated pectin in the diet increased the multiplicity of colon tumors in rats injected with DMH. In another co-carcinogenicity study, carrageenan (15% in the diet) enhanced the incidence of colon tumors in female Fischer 344 rats injected with azoxymethane or *N*-nitrosomethylurea.

Anticarcinogenic effects have been associated with arabinoxylan and inulin in studies involving rats, with glucomannan in mice, and with konjac flour in rats. The antitumor/anticarcinogenic activity of wheat bran arabinoxylan in mice and arabinoxylan-oligosaccharides in rats has also been reported.

In an epidemiology study, a positive correlation between polysaccharide gum consumption and the incidence of mammary carcinoma was found for carrageenan, alginate, agar, and pectin, but not for *sterculia urens* gum.

DISCUSSION

The polysaccharide gums comprise polysaccharides obtained from plants or algae. Based on the different chemical structures of polysaccharide gums, these ingredients can be subdivided into categories such as modified, unmodified, linear, branched, and cyclic. Regardless of how they are categorized, the molecular structures of these ingredients are polymers composed of monosaccharides. Based on chemical similarities, relevant data have been included on analogous polysaccharide ingredients. Therein, inference may be appropriate from one ingredient to the next and from one ingredient to one subgroup of polysaccharides, of which that ingredient or analog is a member.

The substantial molecular sizes of many of these polysaccharides suggest that skin penetration would be unlikely. Specifically, the percutaneous absorption of ¹⁴C-2-hydroxypropyl- β -cyclodextrin through intact hairless mouse skin was extremely low, i.e., approximately 0.02% of the amount applied to the skin. Thus, during cosmetic use, these ingredients are unlikely to have significant systemic bioavailability.

The use concentration data provided indicate that algin is being used in cosmetics at concentrations up to 50% (in mud packs). The Expert Panel acknowledged the absence of skin irritation and sensitization data on algin at this concentration, but noted that results were negative when carboxymethyl inulin was tested at concentrations up to 100% in a skin irritation study involving guinea pigs, and the absence of clinically relevant reactions to polysaccharide gums in dermatologic practice. The Panel is aware of severe granulomatous reactions in patients injected intradermally with an aesthetic injectable filler consisting of purified alginate; however, it was determined that these findings are not relevant to the use of alginates as cosmetic ingredients. Furthermore, systemic toxicity is not a concern in relation to repeated exposure to polysaccharide gums during cosmetic use, considering the absence of gross or microscopic changes in monkeys dosed orally/fed carrageenan in the diet for 7.5 years.

Genotoxicity data for pectin-derived acidic oligosaccharides in mammalian assays were equivocal, but some were classified as clastogenic. However, the Panel noted that clastogenicity was observed only at highly cytotoxic concentrations. The Panel reviewed data indicating that degraded carrageenan (also known as poligeenan) in the diet induced colorectal tumors in rats. Degraded carrageenan used in those studies was produced by acid hydrolysis of a certain type of seaweed. In light of this information and the colon carcinogenicity data, the Panel expressed concern about the use of hydrolyzed carrageenan as a cosmetic ingredient, in the absence of data demonstrating that hydrolyzed carrageenan is chemically dissimilar to poligeenan and does not share its carcinogenic properties. Thus, the Panel determined that method of manufacture and impurities data are needed to determine the safety of hydrolyzed carrageenan in cosmetic products.

Polysaccharide gums are used at concentrations up to 9.5% (avena sativa (oat) starch) in perfumes, at a maximum concentration of 0.45% (corn starch modified) in pump hair sprays, and at concentrations up to 33% (tapioca starch) in powders. The available data indicate that food grade konjac flour (primary polysaccharide component is glucomannan) induced sensory irritation of the respiratory tract in mice and respiratory tract sensitization in guinea pigs. Furthermore, the inhalation of konjac dust in factories in Japan has produced allergic bronchial asthma in sensitized individuals. Additional research suggested that the purified antigen AG40D-2 (acidic protein) was responsible for the respiratory sensitization observed, and that this effect was not attributed to glucomannan. Transbronchial injection of 0.75% carrageenan (in physiological saline) induced pneumonia, followed by emphysema, in rabbits. In consideration of these data, the Panel discussed the potential for incidental inhalation exposures to polysaccharide gums in products that are sprayed or in powder form and agreed that, based on likely airborne particle size distributions and concentrations in the breathing zone and ingredient use, incidental inhalation would not lead to local respiratory effects or systemic effects.

The Panel expressed concern about pesticide residues and heavy metals that may be present in ingredients that are derived from plants. They stressed that the cosmetics industry should continue to use current good manufacturing practices (cGMPs) to limit impurities. The Panel also agreed that the same suggestion is applicable to alkylating and other agents (e.g., haloethylaminopropionic acid; 3-(dodecenyl)-2,5-furandione; and 2,3-epoxypropyltrimethylammonium chloride) that are used to modify polysaccharide gums.

CONCLUSION

The CIR Expert Panel concluded that the following 105 ingredients are safe in the present practices of use and concentration in cosmetics, as described in this safety assessment, and that the available data are insufficient for determining the safety of hydrolyzed carrageenan in cosmetic products.

Linear Polysaccharides and Their Salts

Agar	Alginate Acid	Astragalus Gummiifer Gum
Agarose	Ammonium Alginate*	Calcium Alginate
Algin	Amylose*	Calcium Carrageenan*

Carrageenan
Magnesium Alginate*
Mannan

Polianthes Tuberosa
Polysaccharide
Potassium Alginate

Potassium Carrageenan*
Sodium Carrageenan
TEA-Alginate*

Linear -Modified

Amylodextrin
Hydrolyzed Furcellaran*
Maltodextrin

Sodium Algin Sulfate*

Branched -Unmodified

Amylopectin*
Aphanothece Sacrum
Polysaccharide*
Arabinoxylan*
Avena Sativa (Oat) Starch
Cichorium Intybus (Chicory)
Root Oligosaccharides
Galactarabinan
Ghatti Gum*

Glucomannan
Inulin
Pectin
Phaseolus Angularis Seed
Starch*
Phaseolus Radiatus Seed
Starch*
Pisum Sativum (Pea) Starch*
Pueraria Lobata Starch

Solanum Tuberosum (Potato)
Starch
Starch Acetate
Sterculia Urens Gum
Tamarindus Indica Seed Gum
Tapioca Starch
Triticum Vulgare(Wheat) Starch
Xyloglucan*

Branched - Modified

Calcium Starch
Isododecenylsuccinate*
Calcium Starch
Octenylsuccinate*
Corn Starch Modified
Dextrin
Dextrin Behenate*
Dextrin Isostearate*
Dextrin Laurate*
Dextrin Myristate
Dextrin Palmitate
Dextrin
Palmitate/Ethylhexanoate
Dextrin Stearate*
Glyceryl Alginate
Glyceryl Dimaltodextrin*
Glyceryl Starch
Hydrolyzed Pectin

Hydroxypropyltrimonium
Hydrolyzed Corn Starch
Hydroxypropyltrimonium
Hydrolyzed Wheat Starch
Hydroxypropyl Oxidized
Starch*
Hydroxypropyl Starch
Hydroxypropyltrimonium
Maltodextrin Crosspolymer
Laurdimonium Hydroxypropyl
Hydrolyzed Wheat Starch
Palmitoyl Inulin*
Potassium Dextrin
Octenylsuccinate*
Potassium Undecylenoyl
Alginate*
Potassium Undecylenoyl
Carrageenan*
Potato Starch Modified
Propylene Glycol Alginate
Sodium Carboxymethyl Inulin*
Sodium Carboxymethyl Starch
Sodium Dextrin

Octenylsuccinate*
Sodium Hydrolyzed Potato
Starch Dodecenylsuccinate
Sodium Hydroxypropyl
Oxidized Starch Succinate*
Sodium Oxidized Starch
Acetate/Succinate
Sodium Starch Octenylsuccinate
Sodium/TEA-Undecylenoyl
Carrageenan*
Sodium/TEA-Undecylenoyl
Alginate*
Starch Acetate/Adipate*
Starch Diethylaminoethyl Ether
Starch Hydroxypropyltrimonium
Chloride
Starch Laurate*
Starch Tallowate*
Stearoyl Inulin
Tapioca Starch Crosspolymer*
TEA-Dextrin Octenylsuccinate*
Undecylenoyl Inulin*

Cyclic

Cyclodextrin

Cyclotetraglucose*

Cyclic - Modified

Hydroxyethyl Cyclodextrin
Hydroxypropyl Cyclodextrin
Cyclodextrin Hydroxypropyltrimonium Chloride*

Cyclodextrin Laurate
Methyl Cyclodextrin

Unknown Structural Configuration

Algae Exopolysaccharides*
Cassia Angustifolia Seed Polysaccharide*
Prunus Persica (Peach) Gum*

Unknown Structural Configuration - Modified

Hydrogenated Potato Starch*
Hydrogenated Starch Hydrolysate
Hydrolyzed Corn Starch Hydroxyethyl Ether*
Hydrolyzed Corn Starch Octenylsuccinate
Hydrolyzed Soy Starch*

Hydrolyzed Starch
Hydrolyzed Triticum Spelta Starch*
Hydrolyzed Wheat Starch

*Not reported to be in current use. Were ingredients in this group not in current use to be used in the future, the expectation is that they would be used in product categories and at concentrations comparable to others in this group.

Table 1. Names, CAS Registry Numbers, Definitions and Idealized Structures of the Polysaccharide Gums.¹

[*Italicized text* and all structures below have been added by CIR staff.]

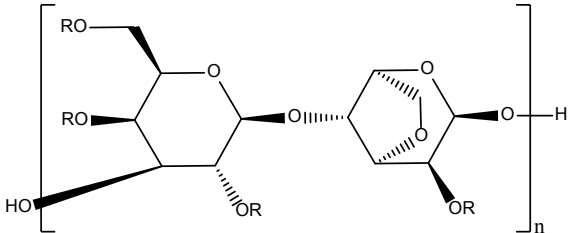
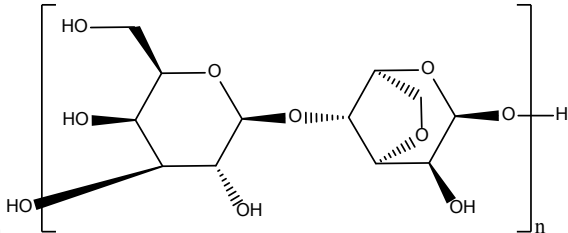
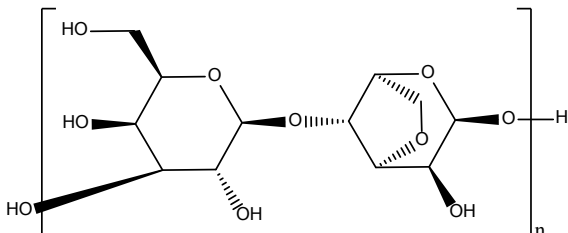
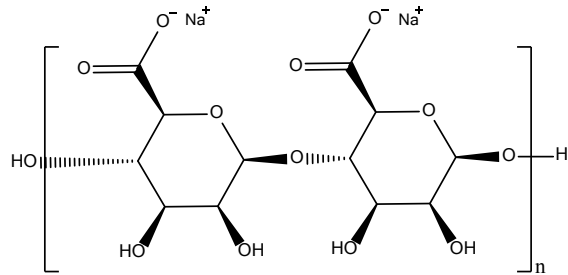
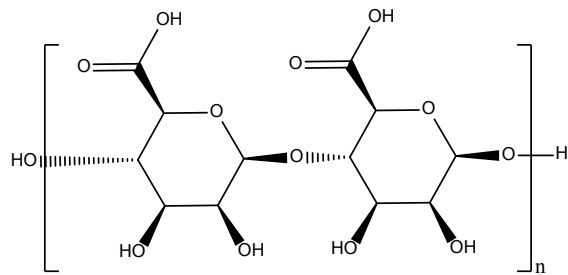
Ingredient CAS No.	Definition	Formula/structure
Linear polysaccharides and their salts		
Agar 9002-18-0	Agar is the dried, hydrophilic, colloidal polygalactoside derived from various Gelidium species or closely related red alga. <i>Agar is typically a mixture of agarose and agarpectin.</i> ⁸⁶	 and  wherein R is hydrogen, sulfate, or pyruvate
Agarose 9012-36-6	Agarose is the polysaccharide extracted from the red seaweed Gracilaria.	
Algin 9005-38-3	Algin is the sodium salt of Alginic Acid. <i>Alginic Acid is the carbohydrate obtained by the alkaline extraction of various species of brown seaweed, Phaeophyceae.</i> Other source: Algin is a linear polymer of anhydro-β-D-mannuronic acid. The main structural feature of this molecule is a chain of 1,4-linked-β-D-mannuronic acid residues. ⁸⁷	
Alginic Acid 9005-32-7	Alginic Acid is the carbohydrate obtained by the alkaline extraction of various species of brown seaweed, Phaeophyceae. <i>Alginic acid is a polysaccharide comprised of 1,4-linked-β-D-mannuronic and α-L-guluronic acids.</i> ⁸⁸	

Table 1. Names, CAS Registry Numbers, Definitions and Idealized Structures of the Polysaccharide Gums.¹

[*Italicized text* and all structures below have been added by CIR staff.]

Ingredient CAS No.	Definition	Formula/structure
Ammonium Alginate 9005-34-9	Ammonium Alginate is the ammonium salt of Alginic Acid. <i>Alginic Acid is the carbohydrate obtained by the alkaline extraction of various species of brown seaweed, Phaeophyceae.</i> Other sources: Alginate, a term that refers to salts and derivatives of alginic acid, is a gelling polysaccharide and a structural component extracted from marine brown algae (<i>Phaeophyceae</i>), in which it is present in the cell wall as water-insoluble salts. ⁸⁹ Alginates are polymers composed of β -1,4-D-mannuronic acid (M) and α -1,4-L-guluronic acid (G). Alginates have been determined to be true block copolymers, organized in homopolymeric blocks consisting of either mannuronate or guluronate, or mixed in heteropolymeric MG-block structures. Alginate, the monovalent salt form of alginic acid, is a non-repeating copolymer that contains two uronic acid monomers, 1,4-linked- β -D-mannuronic and α -L-guluronic acid. ⁹⁰ These residues exist in linear polysaccharide chains that can dimerize to form hydrogels at room temperature in the presence of divalent ions such as calcium.	
Amylose 9005-82-7	Amylose is the carbohydrate stored by plants that consists of a linear (1 $\rightarrow$ 4)-(structure)-D-glucan polymer. Other source: Starch is composed of two polysaccharides, amylose and amylopectin. ⁹¹ Amylose is a complex α -glucan. It is an essentially linear polymer made up of α (1-4)-linked glucopyranose units.	
Astragalus Gummiifer Gum	Astragalus Gummiifer Gum is a dried resinous exudate obtained from Astragalus gummiifer. It is a complex polysaccharide composed of D-galacturonic acid, D-galactose, D-xylose, and L-arabinose, with associated calcium, and potassium cations.	
Calcium Alginate 9005-35-0	Calcium Alginate is the calcium salt of Alginic Acid. <i>Alginic Acid is the carbohydrate obtained by the alkaline extraction of various species of brown seaweed, Phaeophyceae.</i>	
Calcium Carrageenan 9049-05-2	Calcium Carrageenan is the calcium salt of Carrageenan.	
Carrageenan 9000-07-1	Carrageenan is the plant material obtained from various members of the <i>Gigartinaceae</i> or <i>Solieriaceae</i> families of the red seaweed, <i>Rhodophyceae</i> . Other sources: Carrageenan is a high-molecular-weight sulfated polygalactan derived from several species of red seaweeds of the class <i>Rhodophyceae</i> . ³⁵ Native carrageenan is defined as a hydrocolloid isolated from red algae (seaweed) and consisting mainly of varying amounts (depending on the processing methods) of the ammonium, calcium, magnesium, potassium or sodium salts of sulfate esters of galactose and 3,6-anhydrogalactose copolymers (the two hexose units are alternately linked α -1,3 and β -1,4 in the polymer). ⁹² A product called 'degraded carrageenan' has been produced from extracts of <i>Eucheuma spinosum</i> seaweed by treatment with dilute hydrochloric acid. The most common forms of carrageenan are designated as kappa-, iota-, and lambda carrageenans. ⁹³ Kappa carrageenan is mostly the alternating polymer of D-galactose-4-sulfate and 3,6-anhydro-D-galactose. Iota carrageenan is similar, but with the 3,6-anhydro-D-galactose sulfated at the 2-hydroxyl. Between kappa and iota carrageenan, there is a continuum of intermediate compositions that differ only in the degree of sulfation at the 2-OH. Lambda carrageenan has alternating monomeric units composed mostly of D-galactose-2-sulfate (1,3-linked) and D-galactose-2,6-disulfate (1,4-linked).	

Table 1. Names, CAS Registry Numbers, Definitions and Idealized Structures of the Polysaccharide Gums.¹
[Italicized text and all structures below have been added by CIR staff.]

Ingredient CAS No.	Definition	Formula/structure
Magnesium Alginate 37251-44-8	Magnesium Alginate is the magnesium salt of Alginic Acid.	
Mannan 9036-88-8 51395-96-1	Mannan is a natural polysaccharide consisting of a polymer of Mannose.	
Polianthes Tuberosa Polysaccharide	Polianthes Tuberosa Polysaccharide is the polysaccharide fraction produced by the cultured cells of <i>Polianthes tuberosa</i> .	
Potassium Alginate 9005-36-1	Potassium Alginate is the potassium salt of Alginic Acid.	
Potassium Carrageenan 64366-24-1	Potassium Carrageenan is the potassium salt of Carrageenan.	
Sodium Carrageenan 60616-95-7 9061-82-9	Sodium Carrageenan is the sodium salt of Carrageenan.	
TEA-Alginate	TEA-Alginate is the triethanolamine salt of Alginic Acid.	

Table 1. Names, CAS Registry Numbers, Definitions and Idealized Structures of the Polysaccharide Gums.¹
[italicized text and all structures below have been added by CIR staff.]

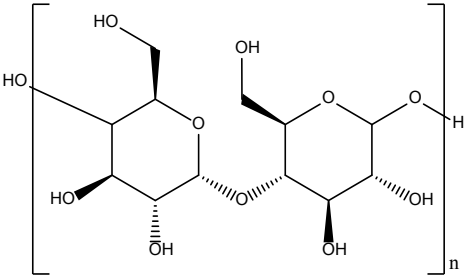
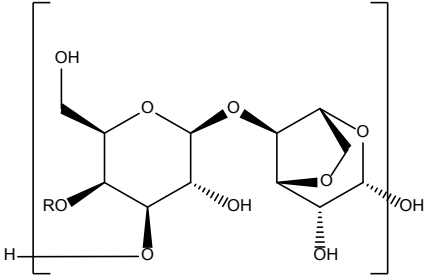
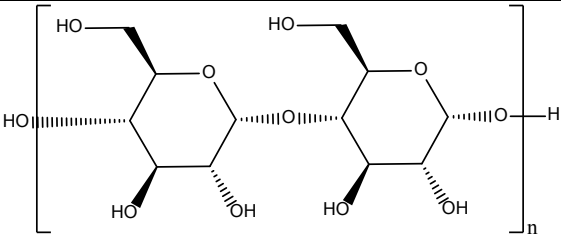
Ingredient CAS No.	Definition	Formula/structure
Linear - modified		
Amylodextrin 9005-84-9	Amylodextrin is the product obtained by treating potato or corn starch with dilute hydrochloric acid.	 The structure shows a repeating unit of amylopectin, a branched polysaccharide. It consists of a central glucose unit linked to several other glucose units via alpha-1,4 and alpha-1,6 glycosidic bonds. The entire unit is enclosed in brackets with a subscript 'n'.
Hydrolyzed Carrageenan 53973-98-1	Hydrolyzed Carrageenan is the hydrolysate of Carrageenan derived by acid, enzyme or other method of hydrolysis.	
Hydrolyzed Furcellaran 73297-69-5	Hydrolyzed Furcellaran is the hydrolysate of furcellaran derived by acid, enzyme or other method of hydrolysis. <i>Furcellaran is composed of D-galactose, 3,6-anhydro-D-galactose and D-galactose- 4-sulfate.</i> Other source: Information relating to the algal source of hydrolyzed furcellaran indicates that this ingredient is a carrageenan (Kappa type) that is obtained from red algae, <i>Furcellaria lumbricallis</i> . ⁷³	 The structure shows a repeating unit of furcellaran, a sulfated polysaccharide. It consists of a central glucose unit linked to several other glucose units via alpha-1,4 and alpha-1,6 glycosidic bonds. The entire unit is enclosed in brackets with a subscript 'n'. Below the structure, it is noted that R is hydrogen or SO₃²⁻. where R is hydrogen or SO₃²⁻
Maltodextrin 9050-36-6	Maltodextrin is the saccharide material obtained by hydrolysis of starch. <i>Maltodextrin is a linear-chain oligosaccharide of glucose, usually obtained from starch by partial, enzymatic treatment.</i> ⁹⁴ The term "maltodextrin" can be applied to any starch hydrolysis product that contains fewer than 20 dextrose (glucose) units linked together.	 The structure shows a repeating unit of maltodextrin, a linear-chain oligosaccharide. It consists of a central glucose unit linked to several other glucose units via alpha-1,4 glycosidic bonds. The entire unit is enclosed in brackets with a subscript 'n'.
Sodium Algin Sulfate 9010-06-4	Sodium Algin Sulfate is the sulfate ester of Algin.	

Table 1. Names, CAS Registry Numbers, Definitions and Idealized Structures of the Polysaccharide Gums.¹

[*Italicized text and all structures below have been added by CIR staff.*]

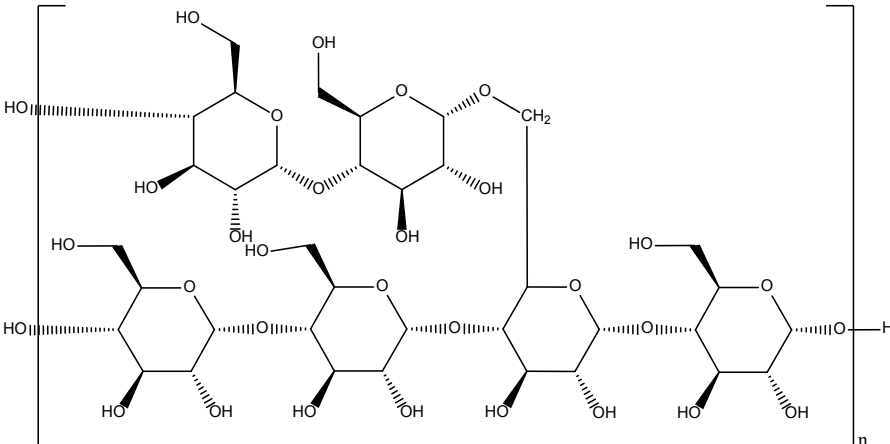
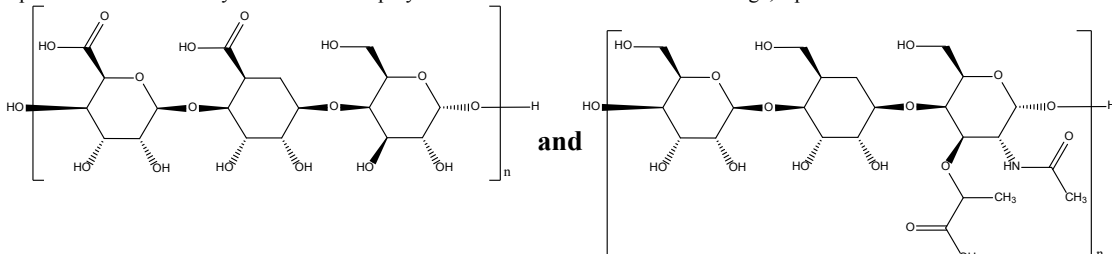
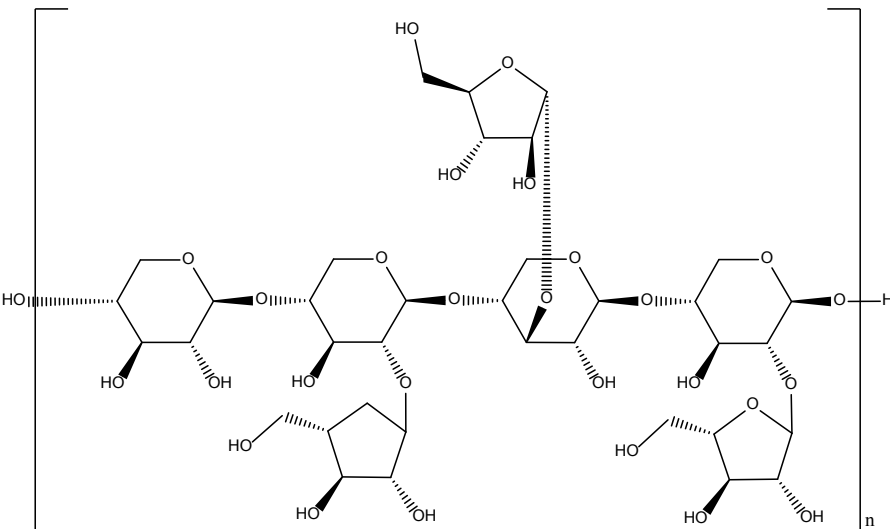
Ingredient CAS No.	Definition	Formula/structure
<i>Branched-unmodified</i>		
Amylopectin 9037-22-3	Amylopectin is the branched chain polysaccharide portion of starch. Other sources: Amylopectin is a complex α -glucan. ⁹¹ It is a highly branched polysaccharide composed of segments of linear $\alpha(1\rightarrow4)$ -linked glucopyranose units joined at branching points via $\alpha(1\rightarrow6)$ glycosidic linkages to give a structure that resembles a dendrimer. Amylopectin consists of numerous short chains of $\alpha(1\rightarrow4)$ -linked D-glucopyranosyl residues with a chain length of approximately 6 to 35 units. ⁹⁵ The chains are $\alpha(1\rightarrow6)$ -linked into clusters defined as groups of chains, in which the internal chain length between the branches is less than 9 residues.	
Aphanothece Sacrum Polysaccharide	Aphanothece Sacrum Polysaccharide is the polysaccharide fraction isolated from the alga, Aphanothece sacrum.	
Arabinoxylan 9040-27-1	Arabinoxylan is a polysaccharide composed of a xylose backbone with arabinose side chains. Other sources: Arabinoxylan is a non-starch polysaccharide, and is also described as a pentosan. ⁹⁶ It can also be sub-categorized as water-extractable arabinoxylan and water-unextractable arabinoxylan. Arabinoxylans consist of D-xylopyranosyl residues, connected together by $\beta(1/4)$ glycosidic bonds. ^{97,98} Moreover, acetic acid, hydroxycinnamic acids, ferulic acid, and p-coumaric acid are linked with xylose residues in arabinoxylan. ^{99,100} The attached moieties are partly or wholly lost when arabinoxylan is extracted from cereal or cereal subfractions using alkaline extraction. ^{96,101,102}	

Table 1. Names, CAS Registry Numbers, Definitions and Idealized Structures of the Polysaccharide Gums.¹
[Italicized text and all structures below have been added by CIR staff.]

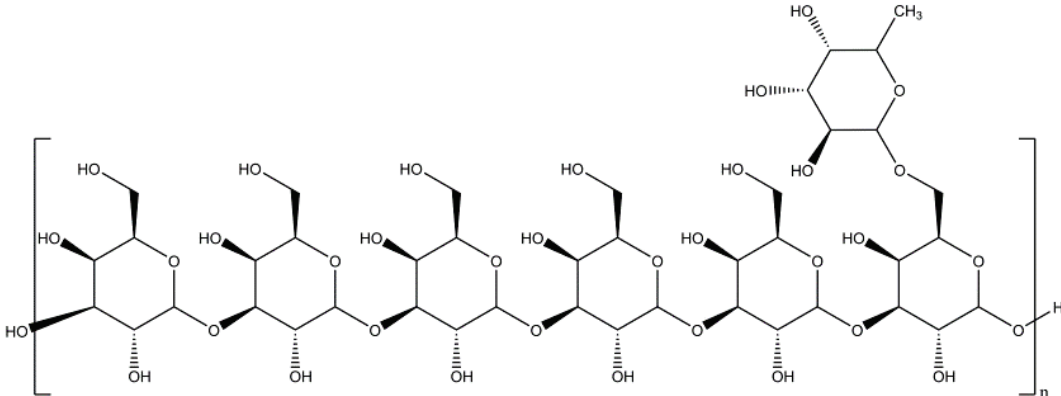
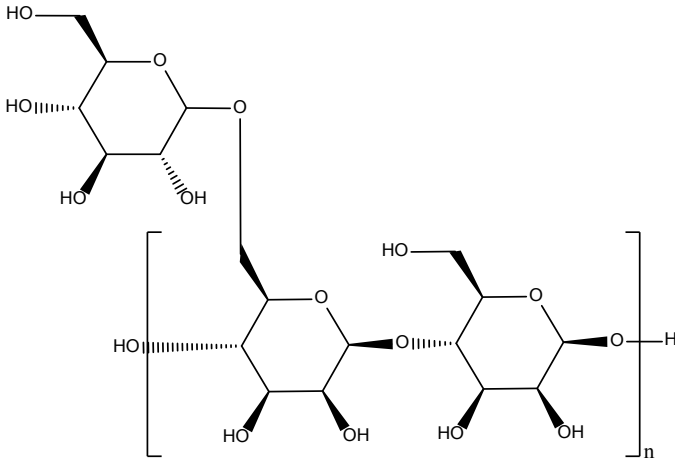
Ingredient CAS No.	Definition	Formula/structure
Avena Sativa (Oat) Starch 9005-25-8 (generic)	Avena Sativa (Oat) Starch is a starch obtained from oats, <i>Avena sativa</i> .	
Cichorium Intybus (Chicory) Root Oligosaccharides	Cichorium Intybus (Chicory) Root Oligosaccharides is the carbohydrate fraction isolated from the roots of <i>Chicorium intybus</i> .	
Galactoarabinan 9036-66-2	Galactoarabinan is the polysaccharide obtained from the extraction of one or more species of the larch tree, <i>Larix</i> . The structure of galactoarabinan is: ¹⁰³	
Ghatti Gum 9000-28-6	Ghatti Gum is the dried, gummy exudate obtained from the stems and bark of <i>Anogeissus latifolia</i> . Other sources: Ghatti gum has been defined as the dried exudate of <i>Anogeissus latifolia</i> . ²⁸ Degradation studies have shown that ghatti gum is a polysaccharide that consists of a backbone of galactose units to which other sugars are attached. ¹⁰⁴ The side chains can consist of arabinose residues and aldobiuronic acids.	
Glucomannan 37220-17-0 11078-31-2 76081-94-2	Glucomannan is the polymer of mannose containing side chains of glucose. Other sources: Glucomannan (a.k.a. konjac flour or konjac mannan) is a β -D-(1 $\rightarrow$ 4)-linked linear copolymer of glucose and mannose substituted with <i>O</i> -acetate every 9-19 sugar units. ¹⁰⁵ It is derived from the tubers of <i>Amorphophallus konjac</i> . Due to the β -glycosidic linkages between the glucose and mannose building blocks (β -1 $\rightarrow$ 4 linkages in the main chain and β -1 $\rightarrow$ 3 linkages at the branch points), glucomannan is commonly regarded as a non-digestible polysaccharide. Additionally, glucomannan contains acetyl groups, approximately one acetyl group per 19 sugar residues. ¹⁰⁶	

Table 1. Names, CAS Registry Numbers, Definitions and Idealized Structures of the Polysaccharide Gums.¹
[Italicized text and all structures below have been added by CIR staff.]

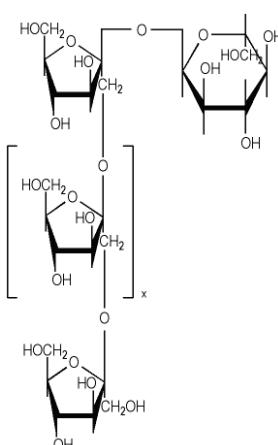
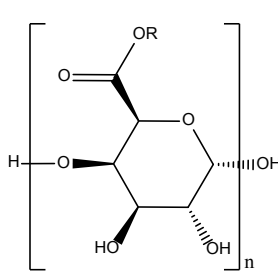
Ingredient CAS No.	Definition	Formula/structure
Inulin 9005-80-5	Inulin is the polysaccharide that conforms to the formula below. Other sources: Inulin has been identified as a fructan, a general term that is used to refer to naturally occurring plant oligo- and polysaccharides. ¹⁰⁷ The term refers to any carbohydrate (linear or branched) in which one or more fructosyl-fructose links constitute the majority of the glycosidic bonds. Within the inulin-type fructans are two general groups of materials, inulin and its subsets, including oligofructose and fructooligosaccharides (FOS). FOS always terminate with a glucose molecule. Oligofructose most often contains only fructose molecules, but may end with a glucose molecule. Inulin is a polydisperse carbohydrate consisting mainly of $\beta(2\rightarrow1)$ fructosyl-fructose links and contains both GF_n and F_m compounds. The n or m represents the number of fructose units (F) linked to each other, which can vary from 2 to 70 with one terminal glucose (G). The terms oligofructose and FOS refer to inulin-type fructans with a maximum average degree of polymerization (DP) less than 10. Additionally, total hydrolysis of inulin yields fructose and glucose. ¹⁰⁷	
Pectin 9000-69-5	Pectin is a purified carbohydrate product obtained from the dilute acid extract of the inner portion of the rind of citrus fruits or from apple pomace. It consists chiefly of partially methoxylated polygalacturonic acids.	 where R is hydrogen or methyl
Phaseolus Angularis Seed Starch	Phaseolus Angularis Seed Starch is a starch obtained from the bean, <i>Phaseolus angularis</i> .	
Phaseolus Radiatus Seed Starch	Phaseolus Radiatus Seed Starch is the starch obtained from the seeds of the bean, <i>Phaseolus radiatus</i> .	
Pisum Sativum (Pea) Starch	Pisum Sativum (Pea) Starch is a starch obtained from <i>Pisum sativum</i> .	
Pueraria Lobata Starch 9005-25-8 (generic)	Pueraria Lobata Starch is the starch obtained from the roots of <i>Pueraria lobota</i> .	
Solanum Tuberosum (Potato) Starch 9005-25-8 (generic)	Solanum Tuberosum (Potato) Starch is a polysaccharide obtained from the potato, <i>Solanum tuberosum</i> .	

Table 1. Names, CAS Registry Numbers, Definitions and Idealized Structures of the Polysaccharide Gums.¹
[Italicized text and all structures below have been added by CIR staff.]

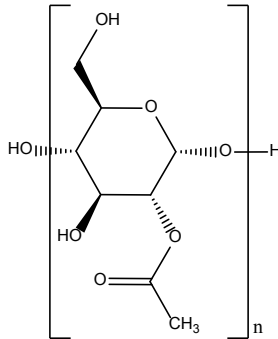
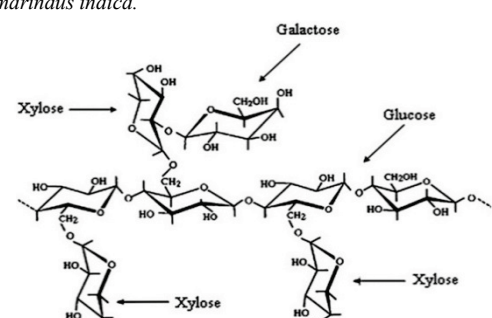
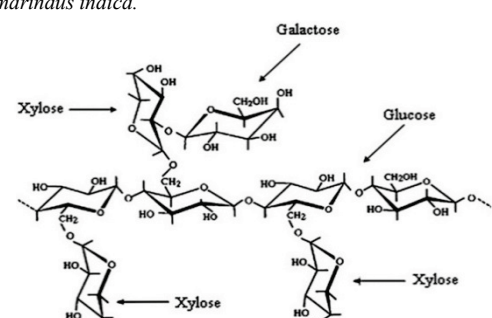
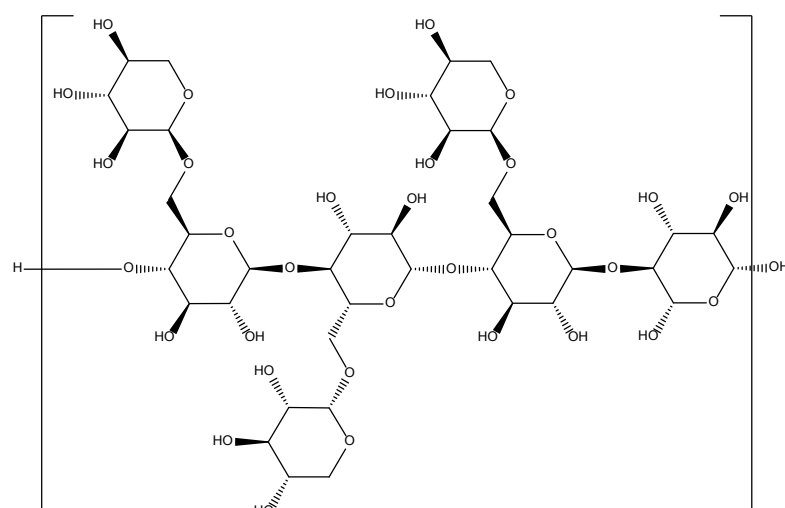
Ingredient CAS No.	Definition	Formula/structure
Starch Acetate 9045-28-7	Starch Acetate is the product obtained by the reaction of acetic acid with starch.	
Sterculia Urens Gum 9000-36-6 [VCRP name: Karaya Gum]	Sterculia Urens Gum is a dried exudate from the tree, <i>Sterculia urens</i> . Other source: Sterculia urens gum (a.k.a. karaya gum), the dried exudate of <i>Sterculia wens</i> Roxb. and other <i>Sterculia</i> spp. (fam. <i>Sterculiaceae</i>), is a complex, partially acetylated polysaccharide with a very high molecular weight. ³⁹ Karaya gum is composed of the sugars galactose, rhamnose, and galacturonic acid.	
Tamarindus Indica Seed Gum 39386-78-2	Tamarindus Indica Seed Gum is the gum obtained from the seeds of <i>Tamarindus indica</i> .	
Tapioca Starch 9005-25-8	Tapioca Starch is the starch obtained from the roots of <i>Manihot esculenta</i> . It consists primarily of amylose and amylopectin.	
Triticum Vulgare (Wheat) Starch 9005-25-8 (generic)	Triticum Vulgare (Wheat) Starch is a starch obtained from wheat, <i>Triticum vulgare</i> .	
Xyloglucan 37294-28-3	Xyloglucan is an oligosaccharide containing a 1,4-β-glucan backbone with 1,6-α-xylosyl residues attached to the 6-position of β-glucosyl residues. Other source: The xyloglucan derived from tamarind seeds is composed of a (1-4)-β-glucan backbone chain, which has (1-6)-α-D-xylose branches that are partially substituted with (1-2)-β-D-galactoxylose. ¹⁰⁸	
Branched – modified (i.e., added sidechains are larger than acetate)		
Calcium Starch Isododecenylsuccinate 194810-88-3	Calcium Starch Isododecenylsuccinate is the calcium salt of the product formed by the reaction of starch with isododecenylsuccinic anhydride.	

Table 1. Names, CAS Registry Numbers, Definitions and Idealized Structures of the Polysaccharide Gums.¹
[Italicized text and all structures below have been added by CIR staff.]

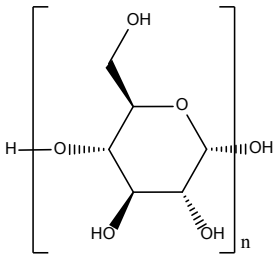
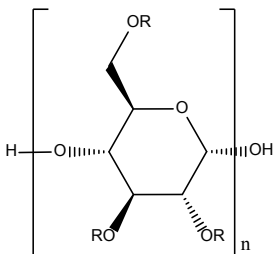
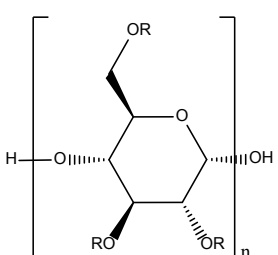
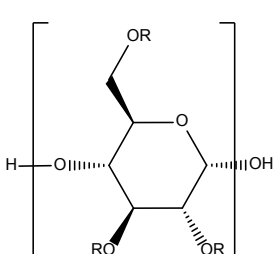
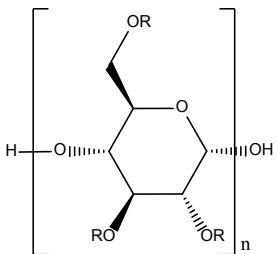
Ingredient CAS No.	Definition	Formula/structure
Calcium Starch Octenylsuccinate	Calcium Starch Octenylsuccinate is the calcium salt of the reaction product of octenylsuccinic anhydride with Zea Mays (Corn) Starch.	
Corn Starch Modified	Corn Starch Modified is the calcium salt of the ester formed from the reaction of 3-(dodecenyl)dihydro-2,5-furandione and corn starch in which the degree of substitution per glucose unit is less than 0.1.	
Dextrin 9004-53-9	Dextrin is a gum produced by the incomplete hydrolysis of starch.	
Dextrin Behenate 112444-74-3	Dextrin Behenate is the ester of Dextrin and Behenic Acid.	 wherein R is the residue of behenic acid
Dextrin Isostearate	Dextrin Isostearate is the ester of Dextrin and Isostearic Acid.	 wherein R is the residue of isostearic acid
Dextrin Laurate 79748-56-4	Dextrin Laurate is the ester of Dextrin and Lauric Acid.	 wherein R is the residue of lauric acid
Dextrin Myristate 93792-77-9	Dextrin Myristate is the ester of Dextrin and Myristic Acid.	 wherein R is the residue of myristic acid

Table 1. Names, CAS Registry Numbers, Definitions and Idealized Structures of the Polysaccharide Gums.¹
[Italicized text and all structures below have been added by CIR staff.]

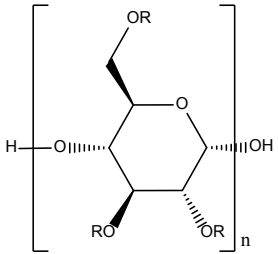
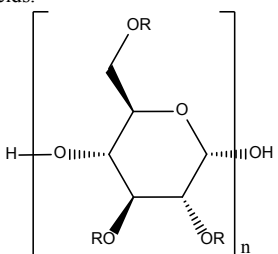
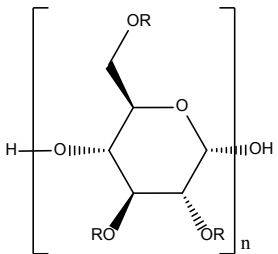
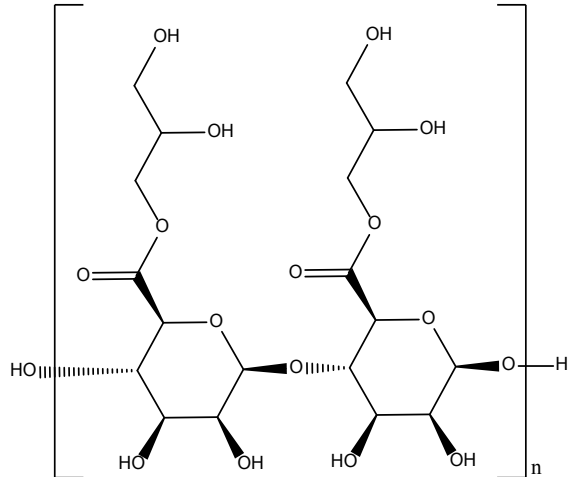
Ingredient CAS No.	Definition	Formula/structure
Dextrin Palmitate 83271-10-7	Dextrin Palmitate is the palmitic acid ester of Dextrin.	 wherein R is the residue of palmitic acid
Dextrin Palmitate/Ethylhexanoate 183387-52-2	Dextrin Palmitate/Ethylhexanoate is the mixed ester of Dextrin with palmitic and ethylhexanoic acids.	 wherein R is the residue of palmitic or ethylhexanoic acid
Dextrin Stearate 37307-33-8	Dextrin Stearate is the ester of Dextrin and Stearic Acid.	 wherein R is the residue of stearic acid
Glyceryl Alginate	Glyceryl Alginate is the ester of glycerin and Alginic Acid.	
Glyceryl Dimaltodextrin	Glyceryl Dimaltodextrin is the reaction product of Glycerin and Maltodextrin.	
Glyceryl Starch	Glyceryl Starch is a partially crosslinked corn starch.	

Table 1. Names, CAS Registry Numbers, Definitions and Idealized Structures of the Polysaccharide Gums.¹
[Italicized text and all structures below have been added by CIR staff.]

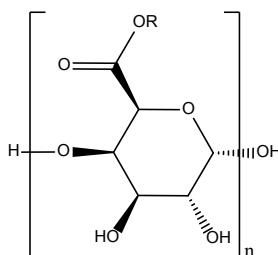
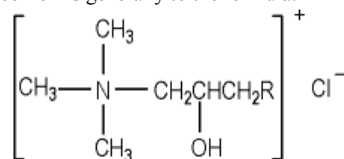
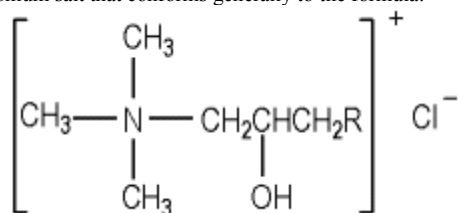
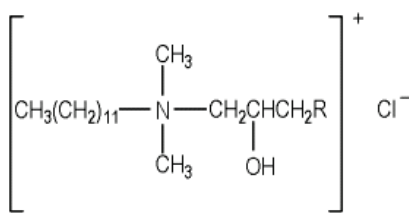
Ingredient CAS No.	Definition	Formula/structure
Hydrolyzed Pectin	Hydrolyzed Pectin is the hydrolysate of Pectin derived by acid, enzyme or other method of hydrolysis. <i>Pectin is a purified carbohydrate product obtained from the dilute acid extract of the inner portion of the rind of citrus fruits or from apple pomace. It consists chiefly of partially methoxylated polygalacturonic acids.</i>	 where R is hydrogen or methyl
Hydroxypropyltrimonium Hydrolyzed Corn Starch	Hydroxypropyltrimonium Hydrolyzed Corn Starch is the quaternary ammonium salt that conforms generally to the formula:	 where R represents the hydrolyzed corn starch moiety.
Hydroxypropyltrimonium Hydrolyzed Wheat Starch	Hydroxypropyltrimonium Hydrolyzed Wheat Starch is the quaternary ammonium salt that conforms generally to the formula:	 where R represents the hydrolyzed wheat starch moiety.
Hydroxypropyl Oxidized Starch	Hydroxypropyl Oxidized Starch is the reaction product of oxygen and Hydroxypropyl Starch.	
Hydroxypropyl Starch 68584-86-1 9049-76-7	Hydroxypropyl Starch is a propylene glycol ether of starch.	
Hydroxypropyltrimonium Maltodextrin Crosspolymer	Hydroxypropyltrimonium Maltodextrin Crosspolymer is a crosslinked polymeric quaternary ammonium salt prepared by the reaction of maltodextrin and glycidyltrimethylammonium chloride with epichlorohydrin.	
Laurdimonium Hydroxypropyl Hydrolyzed Wheat Starch	Laurdimonium Hydroxypropyl Hydrolyzed Wheat Starch is the quaternary ammonium chloride that conforms generally to the formula:	 where R represents the hydrolyzed wheat starch moiety.

Table 1. Names, CAS Registry Numbers, Definitions and Idealized Structures of the Polysaccharide Gums.¹
[Italicized text and all structures below have been added by CIR staff.]

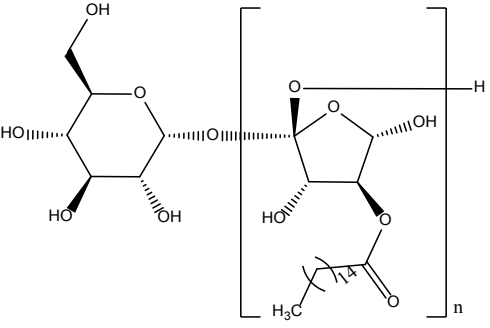
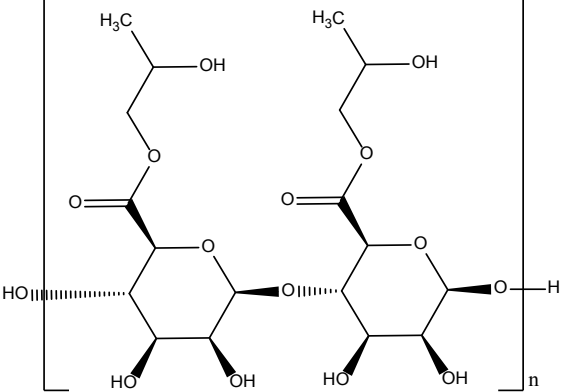
Ingredient CAS No.	Definition	Formula/structure
Palmitoyl Inulin	Palmitoyl Inulin is the condensation product of palmitic acid chloride and the carbohydrate, Inulin.	
Potassium Dextrin Octenylsuccinate	Potassium Dextrin Octenylsuccinate is the potassium salt of the reaction product of octenylsuccinic anhydride with Dextrin.	
Potassium Undecylenoyl Alginate	Potassium Undecylenoyl Alginate is the potassium salt of the condensation product of undecylenic acid chloride and Alginic Acid.	
Potassium Undecylenoyl Carrageenan	Potassium Undecylenoyl Carrageenan is the potassium salt of the condensation product of undecylenic acid chloride and Carrageenan.	
Potato Starch Modified	Potato Starch Modified is the ether formed from the reaction of haloethylaminodipropionic acid and potato starch in which the degree of substitution per glucose unit is less than 0.1.	
Propylene Glycol Alginate 9005-37-2	Propylene Glycol Alginate is a mixture of the propylene glycol esters of alginic acid.	
Sodium Carboxymethyl Inulin 430439-54-6	Sodium Carboxymethyl Inulin is the sodium salt of the product obtained by the reaction of chloroacetic acid with Inulin.	
Sodium Carboxymethyl Starch 9063-38-1	Sodium Carboxymethyl Starch is the sodium salt of a carboxymethyl derivative of starch.	
Sodium Dextrin Octenylsuccinate	Sodium Dextrin Octenylsuccinate is the sodium salt of the reaction product of octenylsuccinic anhydride with Dextrin.	
Sodium Hydrolyzed Potato Starch Dodecenylsuccinate	Sodium Hydrolyzed Potato Starch Dodecenylsuccinate is the sodium salt of the product obtained by the reaction of dextrin with dodecenylsuccinic anhydride.	
Sodium Hydroxypropyl Oxidized Starch Succinate	Sodium Hydroxypropyl Oxidized Starch Succinate is the organic compound that conforms to the formula:	$\text{RO}-\text{CH}_2\text{CH}(\text{OH})\text{CH}_2\text{O}-\text{C}(=\text{O})(\text{CH}_2)_2\text{C}(=\text{O})-\text{ONa}$ where R represents the oxidized starch moiety.
Sodium Oxidized Starch Acetate/Succinate	Sodium Oxidized Starch Acetate/Succinate is the sodium salt of product of the esterification of oxidized starch with acetic acid and succinic acid anhydrides.	

Table 1. Names, CAS Registry Numbers, Definitions and Idealized Structures of the Polysaccharide Gums.¹
[Italicized text and all structures below have been added by CIR staff.]

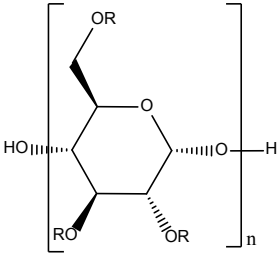
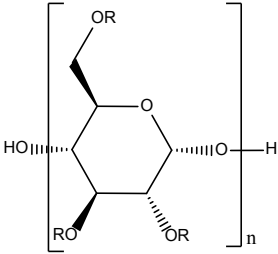
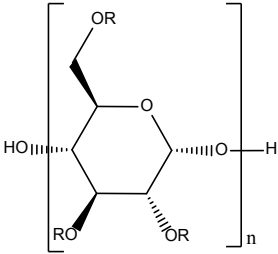
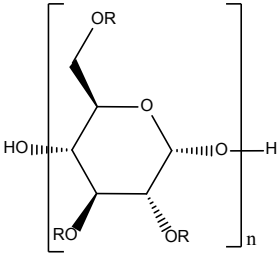
Ingredient CAS No.	Definition	Formula/structure
Sodium Starch Octenylsuccinate 52906-93-1 66829-29-6 70714-61-3	Sodium Starch Octenylsuccinate is the sodium salt of the reaction product of octenylsuccinic anhydride with Zea Mays (Corn) Starch.	
Sodium/TEA-Undecylenoyl Alginate	Sodium/TEA-Undecylenoyl Alginate is the mixed sodium and triethanolamine salt of the condensation product of undecylenic acid chloride and Alginic Acid.	
Sodium/TEA-Undecylenoyl Carrageenan	Sodium/TEA-Undecylenoyl Carrageenan is the mixed sodium and triethanolamine salt of the condensation product of undecylenic acid chloride and Carrageenan.	
Starch Acetate/Adipate 63798-35-6	Starch Acetate/Adipate is the product obtained by the reaction of Zea Mays (Corn) Starch with Adipic Acid and acetic anhydride.	 where R is adipate or acetate
Starch Diethylaminoethyl Ether 9041-94-5	Starch Diethylaminoethyl Ether is the product obtained by conversion of some hydroxyl groups in starch to diethylaminoethyl ether groups.	 where R is hydrogen or constitutes, with the attached oxygen, diethylaminoethyl ether
Starch Hydroxypropyltrimonium Chloride 56780-58-6	Starch Hydroxypropyltrimonium Chloride is the quaternary ammonium compound formed by the reaction of starch with 2,3-epoxypropyltrimethylammonium chloride. Other source: One of the starch hydroxypropyltrimonium chloride trade name materials is defined as an aqueous solution of a naturally derived cationic polysaccharide produced from food grade potato starch. ¹⁰⁹	 where R is hydrogen or constitutes, with the attached oxygen, hydroxypropyltrimonium
Starch Laurate	Starch Laurate is the product obtained by the reaction of lauric acid with starch.	 where R is hydrogen or laurate

Table 1. Names, CAS Registry Numbers, Definitions and Idealized Structures of the Polysaccharide Gums.¹
[Italicized text and all structures below have been added by CIR staff.]

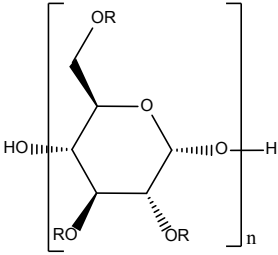
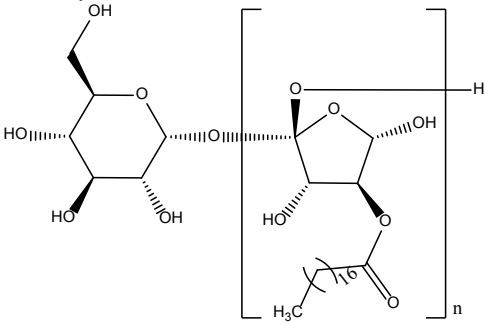
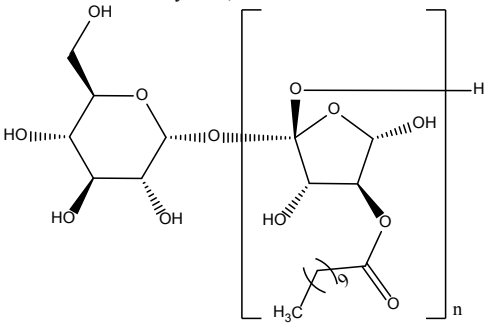
Ingredient CAS No.	Definition	Formula/structure
Starch Tallowate	Starch Tallowate is the ester of starch with the fatty acids derived from Tallow.	 Chemical structure of Starch Tallowate repeating unit: A glucose ring with an OR group at C2 (wedge), HO at C3 (dash), RO at C4 (wedge), and OR at C6 (dash). The structure is enclosed in brackets with a subscript n.
Stearoyl Inulin	Stearoyl Inulin is the condensation product of stearic acid chloride with the carbohydrate, Inulin.	where R is hydrogen or the residue of a fatty acid from tallow  Chemical structure of Stearoyl Inulin repeating unit: A glucose ring with an OH group at C2 (wedge), HO at C3 (dash), HO at C4 (wedge), and HO at C6 (dash). This is linked via an oxygen atom to an inulin unit, which is a fructose ring with an OH group at C2 (wedge), HO at C3 (dash), HO at C4 (wedge), and HO at C6 (dash). The inulin unit is linked to a stearoyl chain, represented as a long alkyl chain with a methyl group at the end. The structure is enclosed in brackets with a subscript n.
Tapioca Starch Crosspolymer	Tapioca Starch Crosspolymer is Tapioca Starch crosslinked with epichlorohydrin.	
TEA-Dextrin Octenylsuccinate	TEA-Dextrin Octenylsuccinate is the triethanolamine salt of the reaction product of octenylsuccinic anhydride with Dextrin.	
Undecylenoyl Inulin	Undecylenoyl Inulin is the condensation product of undecylenic acid chloride with the carbohydrate, Inulin.	 Chemical structure of Undecylenoyl Inulin repeating unit: A glucose ring with an OH group at C2 (wedge), HO at C3 (dash), HO at C4 (wedge), and HO at C6 (dash). This is linked via an oxygen atom to an inulin unit, which is a fructose ring with an OH group at C2 (wedge), HO at C3 (dash), HO at C4 (wedge), and HO at C6 (dash). The inulin unit is linked to an undecylenoyl chain, represented as a long alkyl chain with a methyl group at the end. The structure is enclosed in brackets with a subscript n.

Table 1. Names, CAS Registry Numbers, Definitions and Idealized Structures of the Polysaccharide Gums.¹
[Italicized text and all structures below have been added by CIR staff.]

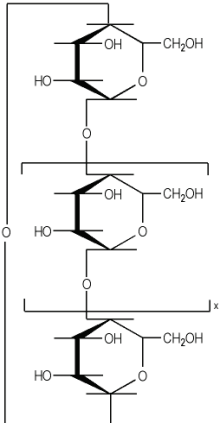
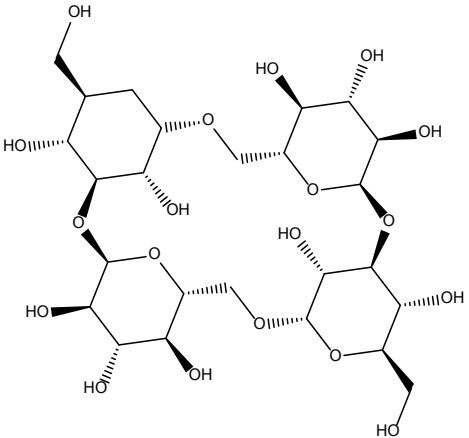
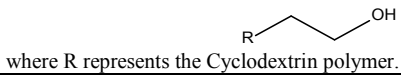
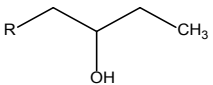
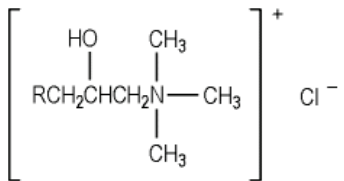
Ingredient CAS No.	Definition	Formula/structure
Cyclic		
Cyclodextrin 12619-70-4 7585-39-9	Cyclodextrin is a cyclic polysaccharide comprised of six to eight glucopyranose units. It conforms to the formula below: Other sources: Cyclodextrins are cyclic amylose-derived oligomers composed of a varying number of α -1-4-linked glucose units. ¹¹⁰ Cyclodextrins contain 6, 7, or 8 glucose units. β -Cyclodextrin is a carbohydrate consisting of seven glucose units. ¹¹¹	 where x may have values from 4 to 6.
Cyclotetraglucose 159640-28-5	Cyclotetraglucose is a cyclic polysaccharide comprised of four Glucose units.	
Cyclic - modified		
Hydroxyethyl Cyclodextrin	Hydroxyethyl Cyclodextrin is the hydroxyethyl ether of Cyclodextrin.	 where R represents the Cyclodextrin polymer.
Hydroxypropyl Cyclodextrin 128446-33-3 128446-35-5	Hydroxypropyl Cyclodextrin is a propylene glycol ether of Cyclodextrin.	 where R represents the Cyclodextrin polymer.
Cyclodextrin Hydroxypropyltrimonium Chloride	Cyclodextrin Hydroxypropyltrimonium Chloride is the organic compound that conforms to the formula:	 where R represents the Cyclodextrin polymer.

Table 1. Names, CAS Registry Numbers, Definitions and Idealized Structures of the Polysaccharide Gums.¹
[Italicized text and all structures below have been added by CIR staff.]

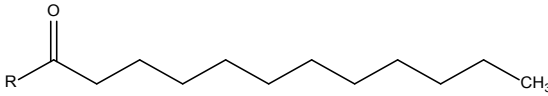
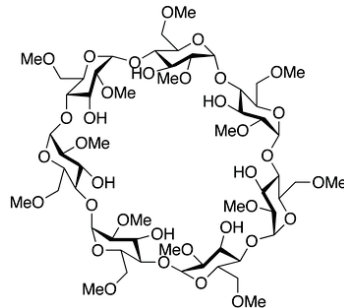
Ingredient CAS No.	Definition	Formula/structure
Cyclodextrin Laurate	Cyclodextrin Laurate is the product obtained by the reaction of Cyclodextrin and lauric acid chloride.	 where R represents the Cyclodextrin polymer.
Methyl Cyclodextrin 128446-36-6	Methyl Cyclodextrin is the product obtained by the methylation of Cyclodextrin.	
Unknown structural configuration		
Algae Exopolysaccharides	Algae Exopolysaccharides (Retired) are exopolysaccharides released by the fermentation of various species of microalgae of the divisions, Rhodophyta and Chlorophyta. The INCI Name, Algae Exopolysaccharides, originally published in 2010, was designated with a retired status in 2015. For an interim period of time, trade name assignments formerly published with the INCI Name Algae Exopolysaccharides will be retained in the retired monograph, and also published with the new name assignment based on the current genus and species name for the specific alga. For further information, consult the Introduction, Retired INCI Names.	
Cassia Angustifolia Seed Polysaccharide	Cassia Angustifolia Seed Polysaccharide is the polysaccharide fraction derived from the seed of <i>Cassia angustifolia</i> . Other source: <i>Cassia angustifolia</i> seed polysaccharide has been defined as a water-soluble galactomannan, consisting of D-galactose and D-mannose in the molar ratio of 3:2, isolated from the seeds of <i>Cassia angustifolia</i> . ¹¹²	
Prunus Persica (Peach) Gum	Prunus Persica (Peach) Gum is the dried, gummy exudate obtained from <i>Prunus persica</i> .	
Unknown structural configuration - modified		
Hydrogenated Potato Starch 68412-29-3 (generic)	Hydrogenated Potato Starch is the end product of the controlled hydrogenation of <i>Solanum Tuberosum</i> (Potato) Starch.	
Hydrogenated Starch Hydrolysate 68425-17-2	Hydrogenated Starch Hydrolysate is the end-product of the controlled hydrogenation of hydrolyzed starch.	
Hydrolyzed Corn Starch Hydroxyethyl Ether	Hydrolyzed Corn Starch Hydroxyethyl Ether is the hydroxyethyl ether of Hydrolyzed Corn Starch.	
Hydrolyzed Corn Starch Octenylsuccinate 125109-81-1	Hydrolyzed Corn Starch Octenylsuccinate is the reaction product of octenylsuccinic anhydride with Hydrolyzed Corn Starch.	
Hydrolyzed Soy Starch 68412-29-3 (generic)	Hydrolyzed Soy Starch is the hydrolysate of soy starch derived by acid, enzyme or other method of hydrolysis.	
Hydrolyzed Starch 34612-38-9 68412-29-3 (generic)	Hydrolyzed Starch is the hydrolysate of starch obtained from <i>Ipomoea batatas</i> , <i>Manihot esculenta</i> , <i>Solanum tuberosum</i> or <i>Zea mays</i> by acid enzyme or other method of hydrolysis.	
Hydrolyzed Triticum Spelta Starch	Hydrolyzed Triticum Spelta Starch is the hydrolysate of the starch obtained from the grain, <i>Triticum spelta</i> derived by acid, enzyme or other method of hydrolysis.	
Hydrolyzed Wheat Starch 68412-29-3 (generic)	Hydrolyzed Wheat Starch is the hydrolysate of wheat starch derived by acid, enzyme or other method of hydrolysis.	

Table 2. Ingredient Functions in Cosmetic Products.¹

<i>Linear polysaccharides and their salts</i>	
Agar	Binders; Fragrance Ingredients; Viscosity Increasing Agents - Aqueous
Agarose	Skin-Conditioning Agents - Humectant; Viscosity Increasing Agents - Aqueous
Algin	Binders; Fragrance Ingredients; Viscosity Increasing Agents - Aqueous
Alginic Acid	Binders; Skin-Conditioning Agents - Miscellaneous; Viscosity Increasing Agents - Aqueous
Ammonium Alginate	Binders; Emulsion Stabilizers; Film Formers; Viscosity Increasing Agents - Aqueous
Amylose	Skin-Conditioning Agents - Humectant
Astragalus Gummiifer Gum	Adhesives; Binders; Emulsion Stabilizers; Film Formers; Fragrance Ingredients; Viscosity Increasing Agents - Aqueous
Calcium Alginate	Fragrance Ingredients; Viscosity Increasing Agents - Aqueous
Calcium Carrageenan	Emulsion Stabilizers; Film Formers; Viscosity Increasing Agents - Aqueous
Carrageenan	Binders; Fragrance Ingredients; Hair Conditioning Agents; Viscosity Increasing Agents - Aqueous
Magnesium Alginate	Binders; Emulsion Stabilizers; Viscosity Increasing Agents - Aqueous
Mannan	Film Formers; Viscosity Increasing Agents - Aqueous
Polianthes Tuberosa Polysaccharide	Skin-Conditioning Agents - Miscellaneous
Potassium Alginate	Binders; Emulsion Stabilizers; Viscosity Increasing Agents - Aqueous
Potassium Carrageenan	Binders; Emulsion Stabilizers; Film Formers; Viscosity Increasing Agents - Aqueous
Sodium Carrageenan	Binders; Emulsion Stabilizers; Film Formers; Viscosity Increasing Agents - Aqueous
TEA-Alginate	Binders; Emulsion Stabilizers; Viscosity Increasing Agents - Aqueous
<i>Linear - modified</i>	
Amylodextrin	Absorbents; Bulking Agents
Hydrolyzed Carrageenan	Skin-Conditioning Agents - Miscellaneous
Hydrolyzed Furcellaran	Skin Protectants
Maltodextrin	Absorbents; Binders; Dispersing Agents - Nonsurfactant; Emulsion Stabilizers; Film Formers; Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous
Sodium Algin Sulfate	Skin-Conditioning Agents - Humectant
<i>Branched – unmodified</i>	
Amylopectin	Binders; Viscosity Increasing Agents - Aqueous
Aphanothece Sacrum Polysaccharide	Absorbents; Emulsion Stabilizers; Film Formers; Viscosity Increasing Agents - Aqueous
Arabinoxylan	Film Formers
Avena Sativa (Oat) Starch	Absorbents
Cassia Angustifolia Seed Polysaccharide	Skin-Conditioning Agents - Emollient
Cichorium Intybus (Chicory) Root	Skin-Conditioning Agents - Miscellaneous
Oligosaccharides	
Galactoarabinan	Film Formers; Fragrance Ingredients
Ghatti Gum	Binders; Emulsion Stabilizers; Surfactants - Emulsifying Agents; Viscosity Increasing Agents - Aqueous
Glucomannan	Skin Protectants; Skin-Conditioning Agents - Miscellaneous
Inulin	Skin-Conditioning Agents - Humectant
Pectin	Binders; Emulsion Stabilizers; Oral Health Care Drugs; Viscosity Increasing Agents - Aqueous
Phaseolus Angularis Seed Starch	Absorbents
Phaseolus Radiatus Seed Starch	Abrasives; Bulking Agents
Pisum Sativum (Pea) Starch	Absorbents; Opacifying Agents; Slip Modifiers

Table 2. Ingredient Functions in Cosmetic Products.¹

Pueraria Lobata Starch	Absorbents; Opacifying Agents; Slip Modifiers
Solanum Tuberosum (Potato) Starch	Absorbents; Binders; Bulking Agents; Viscosity Increasing Agents - Aqueous
Starch Acetate	Hair Conditioning Agents; Skin-Conditioning Agents - Emollient
Sterculia Urens Gum	Adhesives; Binders; Emulsion Stabilizers; Fragrance Ingredients; Hair Fixatives; Viscosity Increasing Agents - Aqueous
Tamarindus Indica Seed Gum	Adhesives; Emulsion Stabilizers; Skin-Conditioning Agents - Humectant; Viscosity Increasing Agents - Aqueous
Tapioca Starch	Viscosity Increasing Agents - Aqueous
Triticum Vulgare (Wheat) Starch	Abrasives; Absorbents; Binders; Bulking Agents; Viscosity Increasing Agents - Aqueous
Xyloglucan	Humectants
<i>Branched – modified (i.e., added sidechains are larger than acetate)</i>	
Calcium Starch Isododecenylsuccinate	Absorbents; Skin-Conditioning Agents - Emollient
Calcium Starch Octenylsuccinate	Absorbents; Emulsion Stabilizers; Viscosity Increasing Agents - Aqueous
Corn Starch Modified	Absorbents; Film Formers; Skin-Conditioning Agents - Miscellaneous; Viscosity Increasing Agents - Nonaqueous
Dextrin	Absorbents; Binders; Bulking Agents; Viscosity Increasing Agents - Aqueous
Dextrin Behenate	Anticaking Agents; Surfactants - Emulsifying Agents
Dextrin Isostearate	Skin-Conditioning Agents - Miscellaneous
Dextrin Laurate	Anticaking Agents; Surfactants - Emulsifying Agents
Dextrin Myristate	Anticaking Agents; Surfactants - Emulsifying Agents
Dextrin Palmitate	Anticaking Agents; Surfactants - Emulsifying Agents
Dextrin Palmitate/Ethylhexanoate	Anticaking Agents; Surfactants - Emulsifying Agents
Dextrin Stearate	Anticaking Agents; Surfactants - Emulsifying Agents
Glyceryl Alginate	Skin-Conditioning Agents - Emollient; Viscosity Increasing Agents - Aqueous
Glyceryl Dimaltodextrin	Humectants; Skin-Conditioning Agents - Humectant
Glyceryl Starch	Absorbents; Binders
Hydrolyzed Pectin	Skin-Conditioning Agents - Miscellaneous
Hydroxypropyltrimonium Hydrolyzed Corn Starch	Antistatic Agents; Film Formers; Hair Conditioning Agents; Hair Fixatives; Hair-Waving/Straightening Agents
Hydroxypropyltrimonium Hydrolyzed Wheat Starch	Antistatic Agents; Hair Conditioning Agents
Hydroxypropyl Oxidized Starch	Film Formers
Hydroxypropyl Starch	Dispersing Agents - Nonsurfactant; Viscosity Increasing Agents - Aqueous
Hydroxypropyltrimonium Maltodextrin Crosspolymer	Dispersing Agents - Nonsurfactant
Laurdimonium Hydroxypropyl Hydrolyzed Wheat Starch	Antistatic Agents; Hair Conditioning Agents
Palmitoyl Inulin	Skin-Conditioning Agents - Emollient; Surfactants - Emulsifying Agents
Potassium Dextrin Octenylsuccinate	Emulsion Stabilizers; Hair Conditioning Agents; Humectants; Skin-Conditioning Agents - Emollient; Surfactants - Emulsifying Agents
Potassium Undecylenoyl Alginate	Emulsion Stabilizers; Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous
Potassium Undecylenoyl Carrageenan	Emulsion Stabilizers; Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous
Potato Starch Modified	Viscosity Increasing Agents - Aqueous
Propylene Glycol Alginate	Binders; Fragrance Ingredients; Viscosity Increasing Agents - Aqueous
Sodium Carboxymethyl Inulin	Chelating Agents; Viscosity Increasing Agents - Aqueous
Sodium Carboxymethyl Starch	Binders; Emulsion Stabilizers; Film Formers; Viscosity Increasing Agents - Aqueous

Table 2. Ingredient Functions in Cosmetic Products.¹

Sodium Dextrin Octenylsuccinate	Emulsion Stabilizers; Hair Conditioning Agents; Humectants; Skin-Conditioning Agents - Emollient; Surfactants - Emulsifying Agents
Sodium Hydrolyzed Potato Starch Dodecenylsuccinate	Surfactants – Foam Boosters
Sodium Hydroxypropyl Oxidized Starch Succinate	Film Formers; Hair Conditioning Agents; Humectants; Skin-Conditioning Agents - Miscellaneous
Sodium Oxidized Starch Acetate/Succinate	Film Formers; Hair Conditioning Agents; Humectants; Skin-Conditioning Agents - Miscellaneous
Sodium Starch Octenylsuccinate	Absorbents; Emulsion Stabilizers; Viscosity Increasing Agents - Aqueous
Sodium/TEA-Undecylenoyl Alginate	Emulsion Stabilizers; Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous
Sodium/TEA-Undecylenoyl Carrageenan	Emulsion Stabilizers; Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous
Starch Acetate/Adipate	Viscosity Increasing Agents - Aqueous
Starch Diethylaminoethyl Ether	Film Formers; Skin-Conditioning Agents - Miscellaneous
Starch Hydroxypropyltrimonium Chloride	Antistatic Agents; Dispersing Agents - Nonsurfactant; Emulsion Stabilizers; Hair Conditioning Agents; Viscosity Increasing Agents - Aqueous
Starch Laurate	Abrasives
Starch Tallowate	Skin-Conditioning Agents - Emollient
Stearoyl Inulin	Skin-Conditioning Agents - Emollient; Surfactants - Emulsifying Agents
Tapioca Starch Crosspolymer	Absorbents; Binders
TEA-Dextrin Octenylsuccinate	Emulsion Stabilizers; Hair Conditioning Agents; Humectants; Skin-Conditioning Agents - Emollient; Surfactants - Emulsifying Agents
Undecylenoyl Inulin	Emulsion Stabilizers; Skin-Conditioning Agents - Emollient
<i>Cyclic</i>	
Cyclodextrin	Absorbents; Chelating Agents
Cyclotetraglucose	Binders; Bulking Agents; Skin-Conditioning Agents - Humectant; Viscosity Increasing Agents - Aqueous
<i>Cyclic - modified</i>	
Hydroxyethyl Cyclodextrin	Skin-Conditioning Agents - Miscellaneous
Hydroxypropyl Cyclodextrin	Chelating Agents; Emulsion Stabilizers
Cyclodextrin Hydroxypropyltrimonium Chloride	Film Formers; Skin-Conditioning Agents - Humectant; Viscosity Increasing Agents - Aqueous
Cyclodextrin Laurate	Film Formers; Skin Protectants; Skin-Conditioning Agents - Humectant
Methyl Cyclodextrin	Chelating Agents
<i>Unknown structural configuration</i>	
Algae Exopolysaccharides	Film Formers; Skin Protectants; Skin-Conditioning Agents - Humectant; Slip Modifiers
Prunus Persica (Peach) Gum	Viscosity Increasing Agents - Aqueous
<i>Unknown structural configuration - modified</i>	
Hydrogenated Potato Starch	Viscosity Increasing Agents - Aqueous
Hydrogenated Starch Hydrolysate	Film Formers; Humectants; Oral Care Agents; Skin-Conditioning Agents - Humectant
Hydrolyzed Corn Starch Hydroxyethyl Ether	Emulsion Stabilizers; Humectants; Skin-Conditioning Agents - Humectant; Viscosity Increasing Agents - Aqueous
Hydrolyzed Corn Starch Octenylsuccinate	Absorbents; Binders; Film Formers
Hydrolyzed Soy Starch	Skin-Conditioning Agents - Miscellaneous
Hydrolyzed Starch	Humectants; Skin Protectants; Skin-Conditioning Agents - Humectant
Hydrolyzed Triticum Spelta Starch	Skin-Conditioning Agents - Miscellaneous
Hydrolyzed Wheat Starch	Skin-Conditioning Agents - Humectant

Table 3. Properties and Method of Manufacture of Polysaccharide Gums

<i>Linear Polysaccharides and Their Salts</i>	
<u>Carrageenan</u>	
Average Molecular Weight: > 100,000 Da. ³⁵	Molecular Weight Range: 196,000–257,000 Da. ¹¹³
Stability: Data on carrageenans (in their sodium ion form without co-gelling cations) included κ -carrageenan from <i>Eucheuma cottonii</i> , ι -carrageenan from <i>Eucheuma spinosum</i> , a κ/λ mixture extracted from <i>Chondrus crispus</i> , and a κ/λ hybrid carrageenan from <i>Gigartina radula</i> . Reasonable stability to heating at 75°C down to pH 4, and the rate of depolymerization increases dramatically as the pH decreases from 4 to 3. ι -Carrageenan is the most stable form, while κ -carrageenan has the greatest susceptibility to acid hydrolysis. The carrageenans from <i>Gigartina radula</i> and <i>Chondrus crispus</i> have intermediate stability. ¹¹⁴	
Carrageenan in the presence of co-gelling cations is much more stable than carrageenan in sodium ion form at 37°C. However, at higher temperatures, the carrageenan is in the random coil state and is more susceptible to acid degradation. Studies of the stability of κ -carrageenan in the presence of potassium ions have shown that acid-catalyzed hydrolysis occurs at temperatures between 55°C and 95°C. Degradation was described as a first-order random hydrolysis process. A 25% reduction in molecular weight was produced at pH 3 after 1.4 h at 50°C, and after only 28 seconds at 90°C. At pH 4, a similar reduction in molecular weight was recorded after 8 h at 50°C and after 15 minutes at 90°C. ¹¹⁴	
<u>Inulin</u>	
Method of Manufacture: Extraction from the roots of <i>Cichorium intybus</i> . ¹¹⁵	
<i>Linear - Modified</i>	
<u>Amylodextrin</u>	
Method of Manufacture: Prepared from waxy maize by enzymatic hydrolysis with pullulanase. ¹¹⁶	
<u>Hydrolyzed Furcellaran</u>	
Method of Manufacture: The polymer furcellaran (a carrageenan [κ type]) obtained from <i>Furcellaria lumbricallis</i> is depolymerized by sub-critical CO ₂ with a low percentage of water, and the product is an opalescent liquid (See Figure 2). ⁷³	
<u>Maltodextrin</u>	
Method of Manufacture: Prepared as a white powder or concentrated solution by partial hydrolysis of corn starch, potato starch, or rice starch with suitable acids and enzymes. ¹¹⁷	
<i>Branched - Unmodified</i>	
<u>Arabinoxylan</u>	
Molecular Weight: 65 to 66 kDa (obtained by sedimentation), ¹¹⁸ 800 - 5000 kDa (obtained by gel filtration), ¹¹⁹ and 70 - 1,000 kDa (obtained by gel filtration). ¹²⁰	
<u>Cichorium Intybus (Chicory) Root Oligosaccharides</u>	
Method of Manufacture: Extraction from the roots of <i>Cichorium intybus</i> . ¹¹⁵	
<u>Ghatti Gum</u>	
Molecular Weight: $\approx 8.94 \times 10^7$ Da. ¹²¹	
<u>Glucomannan</u>	
Average Molecular Weight: 1,000,000 Da; between 200,000 and 2,000,000 Da (commercial samples). ¹²²	
Form: biphasic liquid crystal phase in water at 7 weight% concentration; becomes completely anisotropic at >10 weight%. ¹⁰⁶	
Decomposition: Begins to decompose at approximately 250°C; decomposition is complete at 350°C. ¹²²	
Method of Manufacture: Obtained by a dry milling process of thin tuber (<i>Amorphophallus konjac</i>) slices. ¹⁰⁵ Can also be obtained from monocot storage organs other than tubers, such as leaves, bulbs, roots, or seeds. ¹²² Glucomannan is found in specific large-sized idioblast cells located in the protoplast, and raphide crystal bundles of oxalic acid are enveloped in the polysaccharide. During processing, focus is placed on eliminating the protein membrane of these cells and removing the needle-shaped oxalic acid crystals by sieving, to give residual levels of approximately 0.2% for crude powder and lower for refined grades. ¹²²	
<i>Branched - Modified</i>	
<u>Carboxymethyl Inulin</u>	
Method of Manufacture: Synthesized by reacting inulin with the sodium salt of monochloroacetic acid in the presence of sodium hydroxide. ¹²³	

Table 3. Properties and Method of Manufacture of Polysaccharide Gums

Corn Starch Modified

Method of Manufacture: aqueous corn starch slurry reaction with 3-(dodecyl) dihydro-2,5-furandione.^{66,124}

Dextrin

Method of Manufacture: Dilute acid (e.g. HNO₃) is added to native starch, and the starch is pre-dried. Next, pre-dried-starch is roasted at a temperature between 110°C and 150°C until the color of the starch changes to what is described as appropriate whiteness.¹²⁵ Another production method begins with the suspension of starch in water and adjustment of the pH to between 6 and 8. An enzyme (e.g., liquefying-type amylase) is added to the slurry, which is liquefied at 80°C and 90°C. Starch syrup is degraded to an appropriate viscosity, and the enzyme is made inactive. The syrup is purified by diatomite, active-carbon, ion-exchange resin and then dried.¹²⁵

Dextrin Myristate

Form: Powder or particles.⁶⁷

Color: White to pale yellow.⁶⁷

Odor: Odorless or characteristic.⁶⁷

Melting Point/Freezing Point: 50 ~ 150°C.⁶⁷

Flash Point: 210°C.⁶⁷

Solubility: Insoluble in water, methanol, and ethanol; soluble in xylene, benzene, chloroform, and carbon tetrachloride.⁶⁷

Method of Manufacture: An esterification reaction involving 3-methylpyridine (beta-picoline) and dimethylformamide (DMF) is followed by percolation, washing (methanol and water), centrifugation, drying, riddle, and use of magnets. Riddle is defined as a screening or sieving process that removes large particulate material. Magnets are used to remove metal particles.¹²⁶

Dextrin Isostearate

Form: Soft solid.¹²⁷

Color: Colorless to pale yellow.¹²⁷

Odor: Odorless or characteristic.¹²⁷

Melting Point/Freezing Point: 60 ~ 70°C.¹²⁷

Flash Point: > 200°C.¹²⁷

Solubility: Insoluble in water, methanol, and ethanol; soluble in xylene, benzene, chloroform, and carbon tetrachloride.¹²⁷

Method of Manufacture: The method of manufacture for dextrin isostearate begins with an esterification reaction involving 3-methylpyridine (beta-picoline) and n-heptane, followed by percolation, washing (methanol), drying, and filtration.¹²⁸

Dextrin Palmitate

Form: Powder or particles.^{68,69}

Color: White to pale yellow.^{68,69}

Odor: Odorless or characteristic.^{68,69}

Melting Point/Freezing Point: 50 ~ 130°C; 100 ~ 130°C.^{68,69}

Flash Point: 200 ~ 250°C.^{68,69}

Solubility: Insoluble in water, methanol, and ethanol; soluble in xylene, benzene, chloroform, and carbon tetrachloride.^{68,69}

Method of Manufacture: An esterification reaction involving 3-methylpyridine (beta-picoline) and dimethylformamide (DMF) is followed by percolation, washing (methanol and water), centrifugation, drying, riddle, and use of magnets. Riddle is defined as a screening or sieving process that removes large particulate material. Magnets are used to remove metal particles.¹²⁶

Dextrin Palmitate/Ethylhexanoate

Form: Powder or particles.¹²⁹

Color: White to pale yellow.¹²⁹

Odor: Odorless or characteristic.¹²⁹

Melting Onset Temperature: 120°C.¹²⁹

Flash Point: 216°C.¹²⁹

Table 3. Properties and Method of Manufacture of Polysaccharide Gums**Dextrin Palmitate/Ethylhexanoate**

Solubility: Insoluble in water, methanol, and ethanol; soluble in xylene, benzene, chloroform, and carbon tetrachloride.¹²⁹

Method of Manufacture: An esterification reaction involving 3-methylpyridine (beta-picoline) and dimethylformamide (DMF) is followed by percolation, washing (methanol and water), centrifugation, drying, riddle, and use of magnets. Riddle is defined as a screening or sieving process that removes large particulate material. Magnets are used to remove metal particles.¹²⁶

Glyceryl Dimaltodextrin

Method of Manufacture: Production of maltodextrins involves the obtention of products consisting of D-glucose units that are linked primarily by $\alpha(1\rightarrow4)$ bonds and having dextrose equivalents less than 20.¹³⁰

Hydroxypropyl Starch

Method of Manufacture: Sodium sulfate (Na_2SO_4) and sodium hydroxide (NaOH) are dissolved in water, and starch and propylene oxide are added, and heated to 38°C to 42°C . After the reaction is finished, the slurry is neutralized by acid (H_2SO_4). The starch is then dewatered, washed, and dried. The slurry of hydroxyl-propyl starch may also be degraded by an enzyme (e.g., liquefying-type amylase), purified by diatomite and active-carbon, and then dried.¹²⁵

Potato Starch Modified

Method of Manufacture: An aqueous potato starch slurry is reacted with haloethylaminopropionic acid. This reaction is followed by washing, filtration, and drying.⁷⁰

Sodium Dextrin Octenylsuccinate

Method of Manufacture: **Method 1:** The slurry of sodium starch octenylsuccinate is degraded by an enzyme (e.g., liquefying-type amylase), purified by diatomite and active-carbon, and dried. The dried starch film is crushed into a fine powder. **Method 2:** Dextrin solution and octenylsuccinic anhydride are esterified, whereby the pH value is adjusted between 7 and 8 with alkaline (triethanolamine; sodium hydroxide solution, potassium hydroxide solution). The sodium dextrin octenylsuccinate manufactured according to this method is sold as a liquid. **Method 3:** Dextrin solution and octenylsuccinic anhydride are esterified, whereby the pH value is adjusted between 7 and 8 with sodium hydroxide solution. The solution is then dried.¹²⁵

Sodium Hydrolyzed Potato Starch Dodecenylsuccinate

Solubility: Soluble in water (149.5 - 158.2 g/l).¹³¹

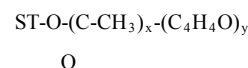
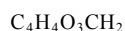
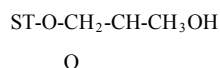
Method of Manufacture: Reaction of a hydrolyzed starch with dodecenylsuccinic anhydride.¹³²

Sodium Hydroxypropyl Oxidized Starch Succinate

Method of Manufacture: Native starch (CAS No. 9005-25-8) and oxidized starch (CAS No. 065996-62-5) can be modified by reacting starch with etherifying and/or esterifying reagents in the presence of an alkaline catalyst.^{15,133}

Reaction to form 2-hydroxypropyl, oxidized starch succinate

Starch 2-Hydroxypropyl Ether, Oxidized + Succinic Anhydride $\rightarrow$ Starch, 2-Hydroxypropyl, Oxidized, Succinic Acid Ester

**Sodium Starch Octenylsuccinate**

Method of Manufacture: Starch is suspended in water, and octenylsuccinic anhydride is added. The slurry is heated to approximately 40°C , and the pH value is adjusted between 6 and 9 with dilute sodium hydroxide solution. The pH value of the solution is stable between 7 and 8, and the slurry is neutralized by acid (H_2SO_4). The starch is then dewatered, washed, and dried. Sodium starch octenylsuccinate may also be suspended in water and dried. The dried starch film is crushed into a fine powder.¹²⁵

Starch Hydroxypropyltrimonium Chloride

Molecular Weight: 2,000,000 Da.¹⁰⁹

Table 3. Properties and Method of Manufacture of Polysaccharide Gums**Starch Hydroxypropyltrimonium Chloride****Form:** Clear to slightly hazy liquid (clear in 1:5 water solution).¹⁰⁹**Dry Substance (%)** 31-33.¹⁰⁹**Color, Gardner:** ≤ 2.5.¹⁰⁹**Odor:** Very mild; slightly sweet.¹⁰⁹**pH @ 20°C:** 3.5-4.5.¹⁰⁹

Method of Manufacture: The starting materials for the production of starch hydroxypropyltrimonium chloride are: oxidized starch and the cationic reagent 3-chloro-2-hydroxypropyltrimethylammonium chloride (CAS No. 3327-22-8).¹³³ The reaction to form cationic starch ether appears below:¹³³

Starch + 2,3-Epoxypropyltrimethylammonium Chloride → Starch Hydroxypropyl Trimethylammonium Chloride

ST-OH CH₂-CH-CH₂-N⁺(CH₃)₃ Cl ST-O-CH₂-CH-CH₂N⁺(CH₃)₃-Cl

According to another source, starch hydroxypropyltrimonium chloride is produced by an aqueous starch slurry reaction with 2,3-epoxypropyltrimethylammonium chloride in the presence of isopropanol. This reaction is followed by washing with isopropanol/water, and the material is then filtered and dried.¹³⁴

Stearoyl Inulin**Form:** Powder or particles.^{71,72}**Color:** White to pale yellow.^{71,72}**Odor:** Odorless or characteristic.^{71,72}**Melting Onset Temperature:** 64°C; 68.2°C.^{71,72}**Flash Point:** 210°C; 214°C.^{71,72}**Solubility:** Insoluble in water, methanol, and ethanol.^{71,72}*Cyclic***Cyclodextrin****Solubility:** Low aqueous solubility (1.85 g/100mL, β-Cyclodextrin).¹³⁵*Unknown Structural Configuration***Algae Exopolysaccharides**

Method of Manufacture: Microalgae is grown in fermenters under conditions that promote the production of the exopolysaccharide, which is secreted by the microalgae. The exopolysaccharides are removed from the cells via filtration or centrifugation, followed by precipitation with alcohol. The exopolysaccharide is then dried and ground to a fine powder. The supplier of this information stated that the CAS number for the ingredient produced (algae exopolysaccharides) is 1122611-69-1, and that the empirical formula for this ingredient is (C₂₇H₄₄O₂₇S)_n. Additionally, it was noted that this is the CAS number for D-galactopyranose.¹³⁶

Cassia Angustifolia Seed Polysaccharide**Average Molecular Weight:** 9.66 x 10⁴ Da.¹³⁷*Unknown Structural Configuration - Modified***Hydrolyzed Starch**

Method of Manufacture: Raw Material (Starch) → Starch slurry → Liquefaction by thermostable α-amylase → Saccharification by isoamylase (to debranch starch amylose) and exomaltotetraohydrolase (to produce maltotetraose) → Heat treatment (inactivation of enzymes) → Filtration → Concentration → Decoloration → Filtration → Storage → Filling and weighing → Hydrolyzed starch.^{138,139}

Table 4. Composition/Impurities Data on Polysaccharide Gums*Linear Polysaccharides and Their Salts***Algin**

After exhaustive methylation of alginic acid, reduction to the corresponding mannoside derivative, and hydrolysis, chromatographic separation indicated that the hydrolyzate contained 88% 2,3-dimethylmannose, 4.5% monomethylmannose, 1% 2,3,4-trimethylmannose, and 6% dimethylglucose.⁸⁷

Carrageenan

The low-molecular-weight forms of carrageenan are <5% of the total composition of the commercial product.³⁵

Twenty-nine samples of food-grade refined carrageenan were analyzed using high-performance liquid gel permeation chromatography. Each sample had no obvious peak of poligeenan (which is defined as degraded carrageenan, detection limit $\approx$ 5%).¹⁴⁰ Poligeenan is produced by a different manufacturing process of seaweed that involves intentional extensive acid hydrolysis, resulting in sulfated galactose polymers with a weight average molecular weight of $\sim$ 15,000 Da.³⁵ Furthermore, according to another source, the molecular weight of poligeenan is in the range of 10,000 to 20,000 Da.¹⁴¹

Inulin

According to the *Food Chemicals Codex*, inulin should contain no more than the following: 1 mg/kg lead, 0.2% ash, and 15% (combined) of monosaccharides (as fructose and glucose) and disaccharides (as sucrose), calculated on the dried basis.¹¹⁵

*Linear - Modified***Hydrolyzed Furcellaran (Mixtures).**^{73,142}

Mixture 1: Components: hydrolyzed furcellaran (0.6%), concentrate of sea water (0.05%), phenoxyethanol (1%), and water (98.35%). **Impurities:** contains heavy metals at a concentration < the limit of quantification, except for Cr (4.74 mg/kg), Ni (1.93 mg/kg), Pb (0.23 mg/kg), Co (0.17 mg/kg), and As (0.11 mg/kg); contains iodine at a concentration < the limit of quantification (i.e., 1 ppm); contains polychlorobiphenyl (PCB) at a concentration < the limit of quantification (i.e., 2 μ g/kg) and research pesticides at a concentration < the limit of quantification (i.e., 10 ng/g).

Mixture 2: Components: hydrolyzed furcellaran (1.35%), phenoxyethanol (1%), and water (97.65%)

Mixture 3: Components: hydrolyzed furcellaran (1.90%), citric acid (0.05%), potassium sorbate (0.10%), and water 97.95%). **Impurities:** contains heavy metals at a concentration < the limit of quantification, except for Cr (0.162 mg/kg) and Pb (0.08 mg/kg); contains iodine at a concentration < the limit of quantification (i.e., 9 ppm); contains PCB at a concentration < the limit of quantification (i.e., 10 μ g/kg) and research pesticides at a concentration < the limit of quantification (i.e., 10 ng/g).

Maltodextrin

According to the *Food Chemicals Codex*, maltodextrin should contain no more than the following: 0.5 mg/kg lead, 0.0025% sulfur dioxide, 1% maltodextrins produced from high-amylose starches, and 0.5% all other types of maltodextrins.¹¹⁵

*Branched - Unmodified***Arabinoxylan**

Arabinoxylans are complex, as the side branches of the main chain arabinose and xylose units contain small amounts of xylopyranose, galactopyranose, and α -D-glucuronic acid or 4-O-methyl- α -D-glucuronic acid.¹⁴³

Glucomannan

Konjac flour consists of the following: carbohydrates (as water-soluble fiber, $\sim$ 75% of glucomannan composition), protein (2-8%), fat (<1%), ash (3-5%), and moisture (<15%).¹⁰⁵

Sterculia Urens Gum

Commercial sterculia urens gum contains 19%-21% of rhamnose and similar proportions of galactose and galacturonic acid.³⁶ Nitrogen content (probably non-protein in nature) of 0.07% has also been reported.⁵¹

*Branched - Modified***Dextrin Myristate**

Dextrin myristate contains: dextrin myristate (> 95%); moisture, based on loss of drying (< 1%); myristic acid (< 5%); 3-Methylpyridine (beta-picoline) (< 300 ppm); DMF (< 5 ppm, detection limit); and methanol (< 5 ppm, detection limit).¹⁴⁴

Dextrin Palmitate

Dextrin palmitate contains: dextrin palmitate (> 95%); moisture, based on loss on drying (< 1%); palmitic acid (< 5%); 3-methylpyridine (beta-picoline) (< 300 ppm; < 1,000 ppm); DMF (< 5 ppm, detection limit); and methanol (< 5 ppm, detection limit).^{145,146}

Dextrin Palmitate/Ethylhexanoate

Dextrin Palmitate/Ethylhexanoate contains: dextrin palmitate/ethylhexanoate (> 95%); moisture, based on loss on drying (< 3%); palmitic acid and 2-ethylhexanoic acid (< 5%); 3-Methylpyridine (beta-picoline) (< 300 ppm); DMF (< 5 ppm, detection limit); and methanol (< 5 ppm).¹⁴⁷

Table 4. Composition/Impurities Data on Polysaccharide Gums**Dextrin Isostearate**

Dextrin isostearate contains: dextrin isostearate (> 95%); isostearic acid (< 5%); 3-methylpyridine (beta-picoline) (< 300 ppm); heptane (< 200 ppm); and methanol (< 5 ppm, detection limit).¹⁴⁸

Sodium Hydrolyzed Potato Starch Dodecenylsuccinate

Impurities: antimony (7.53 mg/kg), arsenic (< 2 mg/kg), barium (0.271 mg/kg), cadmium (< 0.2 mg/kg), chromium (< 0.25 mg/kg), cobalt (< 1.5 mg/kg), copper (< 0.25 mg/kg), lead (< 1.5 mg/kg), nickel (< 1 mg/kg), selenium (< 4.86 mg/kg), zinc (1.49 mg/kg), and mercury (< 0.1 mg/kg).¹⁴⁹

Starch Hydroxypropyltrimonium Chloride

Starch hydroxypropyltrimonium chloride consists of approximately 30% solids, and is preserved with food grade sodium benzoate.¹⁰⁹

Impurities/residuals data: diol levels (< 2%), enol levels (< 1.5%), and quaternizing agent (< 0.1%).¹³⁴

Stearoyl Inulin

Stearoyl inulin contains: stearoyl inulin (> 95%); moisture, based on loss on drying (< 1%); stearic acid (< 5%); 3-Methylpyridine (beta-picoline) (< 300 ppm); DMF (< 5 ppm, detection limit); and methanol (< 5 ppm, detection limit).¹⁵⁰

Unknown Structural Configuration

Cassia Angustifolia Seed Polysaccharide

The purified seed galactomannan contains mannose:galactose in a ratio of 2.90:1.¹³⁷

Unknown Structural Configuration – Modified

Hydrolyzed Starch

Composition/Properties data on two hydrolyzed starch products are available (See Table 5).^{138,139}

Table 5. Composition/Properties Data on Two Hydrolyzed Starch (unknown structural configuration – modified) Products.^{138,139}

Product 1	Product 2
G1 (glucose): 2% (not more than 5% for the specification)	G1 (glucose): 2.5% (not more than 5% for the specification)
G2 (maltose): 7%	G2 (maltose): 6%
G3 (maltotriose)*: 10%	G3 (maltotriose)*: 9.5%
G4 (maltotetraose)**: 53% (not less than 50% for the specification)	G4 (maltotetraose)**: 74% (not less than 70% for the specification)
G5 (maltopentaose***): 2%	G5 (maltopentaose***): 0.5%
≥ G6****: 26%	≥ G6****: 8%
Loss on drying (water content): ≈ 25% (solids specification: not less than 74%)	Loss on drying (water content): ≈ 28% (solids specification: not less than 72%)
Residue on ignition: ≤ 0.05%	Residue on ignition: ≤ 0.05%
Heavy metals (as lead): ≤ 5 ppm	Heavy metals (as lead): ≤ 5 ppm
Arsenic (as As ₂ O ₃): ≤ 2 ppm	Arsenic (as As ₂ O ₃): ≤ 2 ppm

*O-α-glucopyranosyl-(1→4)-O-α-D-glucopyranosyl-(1→4)-D-glucose (maltotriose)

**O-α-glucopyranosyl-[(1→4)-O-α-D-glucopyranosyl]₂-(1→4)-D-glucose (maltotetraose)

***O-α-glucopyranosyl-[(1→4)-O-α-D-glucopyranosyl]₃-(1→4)-D-glucose (maltopentaose)

****O-α-glucopyranosyl-[(1→4)-O-α-D-glucopyranosyl]_n-(1→4)-D-glucose (n ≥ 4)

Table 6. Current Frequency and Concentration of Use According to Duration and Type of Exposure.^{16,17,18,19}

	Maltodextrin		Glucomannan		Agar	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. Range	542	0.00001-4	NR	0.3-17	67	0.002-1
Duration of Use						
<i>Leave-On</i>	327	0.00001-3	NR	NR	49	0.002-1
<i>Rinse off</i>	188	0.00006-3	NR	0.3-17	17	0.0043-0.015
<i>Diluted for (bath) Use</i>	27	0.22-4	NR	NR	1	NR
Exposure Type						
<i>Eye Area</i>	42	0.001-2.5	NR	17	3	1
<i>Incidental Ingestion</i>	13	0.00075-0.6	NR	NR	NR	NR
<i>Incidental Inhalation- Sprays</i>	189	0.00012-0.38	NR	NR	24	0.0075-1*
<i>Incidental Inhalation- Powders</i>	178	0.005-1	NR	NR	25	0.0075**
<i>Dermal Contact</i>	377	0.00001-4	NR	0.3-17	64	0.002-1
<i>Deodorant (underarm)</i>	NR	0.0045-0.12	NR	NR	NR	NR
<i>Hair - Non-Coloring</i>	80	0.00012-2	NR	NR	3	1
<i>Hair-Coloring</i>	65	0.0001-0.0033	NR	NR	NR	NR
<i>Nail</i>	NR	0.0015-3	NR	NR	NR	NR
<i>Mucous Membrane</i>	80	4	NR	NR	5	NR
<i>Baby Products</i>	2	NR	NR	NR	NR	NR
	Agarose		Algin		Alginic Acid	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. Range	10	0.2-0.7	326	0.001-50	13	NR
Duration of Use						
<i>Leave-On</i>	10	0.2-0.7	194	0.001-18	12	NR
<i>Rinse off</i>	NR	NR	131	0.01-50	1	NR
<i>Diluted for (bath) Use</i>	NR	NR	1	0.1	NR	NR
Exposure Type						
<i>Eye Area</i>	NR	NR	40	0.025-0.75	3	NR
<i>Incidental Ingestion</i>	NR	NR	NR	1.1	NR	NR
<i>Incidental Inhalation- Sprays</i>	1	NR	111	0.001-0.025	6	NR
<i>Incidental Inhalation- Powders</i>	1	NR	119	0.025	6	NR
<i>Dermal Contact</i>	10	0.2-0.7	315	0.001-50	13	NR
<i>Deodorant (underarm)</i>	9	0.7	NR	0.001	NR	NR
<i>Hair - Non-Coloring</i>	NR	NR	3	0.001-0.05	NR	NR
<i>Hair-Coloring</i>	NR	NR	1	1.3	NR	NR
<i>Nail</i>	NR	NR	1	0.002	NR	NR
<i>Mucous Membrane</i>	NR	NR	3	0.01-1.1	NR	NR
<i>Baby Products</i>	NR	NR	4	NR	1	NR
	Amylodextrin		Astragalus Gummiifer Gum		Avena Sativa (Oat) Starch	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. Range	2	0.00004	7	NR	5	0.1-9.5
Duration of Use						
<i>Leave-On</i>	2	NR	5	NR	3	0.1-9.5
<i>Rinse off</i>	NR	0.00004	2	NR	2	3.6
<i>Diluted for (bath) Use</i>	NR	NR	NR	NR	NR	NR
Exposure Type						
<i>Eye Area</i>	NR	NR	NR	NR	NR	NR
<i>Incidental Ingestion</i>	NR	NR	NR	NR	NR	NR
<i>Incidental Inhalation- Sprays</i>	1	NR	3	NR	2	0.1-9.5
<i>Incidental Inhalation- Powders</i>	1	NR	3	NR	3	0.1
<i>Dermal Contact</i>	2	NR	4	NR	5	0.1-9.5
<i>Deodorant (underarm)</i>	NR	NR	NR	NR	NR	NR
<i>Hair - Non-Coloring</i>	NR	0.00004	2	NR	NR	NR
<i>Hair-Coloring</i>	NR	NR	1	NR	NR	3.6
<i>Nail</i>	NR	NR	NR	NR	NR	NR
<i>Mucous Membrane</i>	NR	NR	NR	NR	NR	NR
<i>Baby Products</i>	NR	NR	NR	NR	NR	NR

Table 6. Current Frequency and Concentration of Use According to Duration and Type of Exposure.^{16,17,18}

	Calcium Alginate		Carrageenan		Cassia Angustifolia Seed Polysaccharide	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. Range	9	0.01-3	249	0.003-15.7	36	0.002-0.75
Duration of Use						
<i>Leave-On</i>	9	0.01-3	181	0.003-15.7	35	0.002
<i>Rinse off</i>	NR	0.01	63	0.003-3.7	1	0.025-0.75
<i>Diluted for (bath) Use</i>	NR	NR	5	0.1-3	NR	NR
Exposure Type						
<i>Eye Area</i>	NR	NR	18	0.2-3.7	3	NR
<i>Incidental Ingestion</i>	NR	NR	25	1-1.1	3	0.002
<i>Incidental Inhalation- Sprays</i>	2	0.016-1	118	0.03-15.7*	15	0.0025*-0.075*
<i>Incidental Inhalation- Powders</i>	3	0.4-3	11	NR	21	0.0025**-0.025**
<i>Dermal Contact</i>	9	0.01-3	206	0.003-3.7	33	0.0025-0.025
<i>Deodorant (underarm)</i>	NR	0.016-1	NR	NR	NR	NR
<i>Hair - Non-Coloring</i>	NR	NR	14	0.003-15.7	NR	0.025-0.75
<i>Hair-Coloring</i>	NR	NR	NR	NR	NR	NR
<i>Nail</i>	NR	NR	2	NR	NR	NR
<i>Mucous Membrane</i>	NR	NR	35	0.1-3	3	0.002
<i>Baby Products</i>	NR	NR	1	NR	NR	NR
	Cichorium Intybus (Chicory) Root Oligosaccharides		Corn Starch Modified		Cyclodextrin	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. Range	2	NR	86	0.0062-45.7	128	0.000025-4
Duration of Use						
<i>Leave-On</i>	2	NR	75	0.12-45.7	101	0.000025-4
<i>Rinse off</i>	NR	NR	10	0.0062-3	26	0.0042-1.6
<i>Diluted for (bath) Use</i>	NR	NR	1	9	1	NR
Exposure Type						
<i>Eye Area</i>	NR	NR	7	0.9-8	19	0.05-0.25
<i>Incidental Ingestion</i>	NR	NR	2	0.4	2	0.1
<i>Incidental Inhalation- Sprays</i>	2	NR	48	0.45-45.7*	69	0.08-2.5
<i>Incidental Inhalation- Powders</i>	2	NR	33	0.44**-15	59	0.2
<i>Dermal Contact</i>	2	NR	59	0.0062-15	118	0.0005-4
<i>Deodorant (underarm)</i>	NR	NR	NR	0.12	NR	2.5-4
<i>Hair - Non-Coloring</i>	NR	NR	17	0.45-45.7	5	0.000025-1.6
<i>Hair-Coloring</i>	NR	NR	4	NR	3	NR
<i>Nail</i>	NR	NR	NR	NR	NR	NR
<i>Mucous Membrane</i>	NR	NR	6	0.0062-9	4	0.1-0.73
<i>Baby Products</i>	NR	NR	2	NR	NR	NR
	Cyclodextrin Laurate		Dextrin		Dextrin Myristate	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. Range	5	0.0035	177	0.000008-43	NR	0.05-19
Duration of Use						
<i>Leave-On</i>	5	0.0035	159	0.000008-30	NR	0.094-19
<i>Rinse off</i>	NR	NR	18	0.001-43	NR	0.05-7
<i>Diluted for (bath) Use</i>	NR	NR	NR	5	NR	NR
Exposure Type						
<i>Eye Area</i>	2	NR	21	0.000008-30	NR	0.094-19
<i>Incidental Ingestion</i>	NR	NR	1	0.008	NR	7-15
<i>Incidental Inhalation- Sprays</i>	3	NR	95	0.00037-2.8	NR	0.099-18
<i>Incidental Inhalation- Powders</i>	3	0.0035**	96	0.0044-2.8	NR	0.3**-16**
<i>Dermal Contact</i>	5	0.0035	168	0.000008-43	NR	0.05-19
<i>Deodorant (underarm)</i>	NR	NR	NR	NR	NR	NR
<i>Hair - Non-Coloring</i>	NR	NR	2	0.00026-0.001	NR	0.099-1
<i>Hair-Coloring</i>	NR	NR	2	NR	NR	NR
<i>Nail</i>	NR	NR	4	0.2	NR	NR
<i>Mucous Membrane</i>	NR	NR	3	0.008-5	NR	7-15
<i>Baby Products</i>	NR	NR	NR	NR	NR	NR

Table 6. Current Frequency and Concentration of Use According to Duration and Type of Exposure.^{16,17,18}

	Dextrin Palmitate		Dextrin Palmitate/Ethylhexanoate		Dextrin Palmitate/Stearate	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. Range	77	0.0001-16.8	4	NR	NR	0.1-18
Duration of Use						
<i>Leave-On</i>	71	0.0001-16.8	4	NR	NR	0.1-18
<i>Rinse off</i>	6	0.0002-0.0097	NR	NR	NR	NR
<i>Diluted for (bath) Use</i>	NR	NR	NR	NR	NR	NR
Exposure Type						
<i>Eye Area</i>	13	0.0001-2	NR	NR	NR	0.3-18
<i>Incidental Ingestion</i>	37	0.1-16.8	2	NR	NR	4.5-5
<i>Incidental Inhalation- Sprays</i>	5	NR	1	NR	NR	NR
<i>Incidental Inhalation- Powders</i>	5	0.1-0.5**	1	0.1-3**	NR	0.1-3**
<i>Dermal Contact</i>	33	0.0001-13	2	NR	NR	0.1-10
<i>Deodorant (underarm)</i>	NR	NR	NR	NR	NR	NR
<i>Hair - Non-Coloring</i>	NR	NR	NR	NR	NR	NR
<i>Hair-Coloring</i>	NR	NR	NR	NR	NR	NR
<i>Nail</i>	NR	0.025	NR	NR	NR	NR
<i>Mucous Membrane</i>	38	0.1-16.8	2	NR	NR	4.5-5
<i>Baby Products</i>	NR	NR	NR	NR	NR	NR
	Galactoarabinan		Glyceryl Alginate		Glyceryl Starch	
	# of Uses	# of Uses	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. Range	97	NR	NR	0.5	1	4
Duration of Use						
<i>Leave-On</i>	73	NR	NR	0.5	NR	4
<i>Rinse off</i>	24	NR	NR	NR	1	NR
<i>Diluted for (bath) Use</i>	NR	NR	NR	NR	NR	NR
Exposure Type						
<i>Eye Area</i>	21	NR	NR	NR	NR	NR
<i>Incidental Ingestion</i>	2	NR	NR	NR	NR	NR
<i>Incidental Inhalation- Sprays</i>	21	NR	NR	NR	NR	NR
<i>Incidental Inhalation- Powders</i>	21	NR	NR	0.5**	NR	4**
<i>Dermal Contact</i>	76	NR	NR	0.5	1	4
<i>Deodorant (underarm)</i>	NR	NR	NR	NR	NR	NR
<i>Hair - Non-Coloring</i>	9	NR	NR	NR	NR	NR
<i>Hair-Coloring</i>	NR	NR	NR	NR	NR	NR
<i>Nail</i>	NR	NR	NR	NR	NR	NR
<i>Mucous Membrane</i>	5	NR	NR	NR	NR	NR
<i>Baby Products</i>	NR	NR	NR	NR	NR	NR
	Hydrogenated Starch Hydrolysate		Hydrolyzed Corn Starch Octenylsuccinate		Hydrolyzed Pectin	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. Range	60	0.00007-3.8	13	0.06-0.67	14	NR
Duration of Use						
<i>Leave-On</i>	41	0.00007-0.75	11	0.06	12	NR
<i>Rinse off</i>	19	0.13-3.8	2	0.18-0.67	2	NR
<i>Diluted for (bath) Use</i>	NR	NR	NR	NR	NR	NR
Exposure Type						
<i>Eye Area</i>	1	0.00007-0.5	NR	NR	1	NR
<i>Incidental Ingestion</i>	1	0.065-3.8	NR	NR	NR	NR
<i>Incidental Inhalation- Sprays</i>	33	3.8*	7	NR	10	NR
<i>Incidental Inhalation- Powders</i>	29	0.0007**-0.54**	7	NR	10	NR
<i>Dermal Contact</i>	49	0.00007-0.75	13	0.06-0.67	14	NR
<i>Deodorant (underarm)</i>	NR	NR	3	NR	NR	NR
<i>Hair - Non-Coloring</i>	10	0.13	NR	NR	NR	NR
<i>Hair-Coloring</i>	NR	NR	NR	NR	NR	NR
<i>Nail</i>	NR	NR	NR	NR	NR	NR
<i>Mucous Membrane</i>	2	0.065-3.8	NR	0.67	NR	NR
<i>Baby Products</i>	NR	NR	NR	NR	NR	NR

Table 6. Current Frequency and Concentration of Use According to Duration and Type of Exposure.^{16,17,18}

	Hydrolyzed Starch		Hydrolyzed Wheat Starch		Hydroxyethyl Cyclodextrin	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. Range	NR	0.000013-0.00046	274	0.000003-0.31	NR	1.2
Duration of Use						
<i>Leave-On</i>	NR	0.00046	118	0.00005-0.31	NR	1.2
<i>Rinse off</i>	NR	0.000013	156	0.000003-0.25	NR	NR
<i>Diluted for (bath) Use</i>	NR	NR	4	0.000003	NR	NR
Exposure Type						
<i>Eye Area</i>	NR	NR	6	0.03-0.038	NR	1.2
<i>Incidental Ingestion</i>	NR	NR	NR	NR	NR	NR
<i>Incidental Inhalation- Sprays</i>	NR	0.00046*	66	0.00005-0.02	NR	NR
<i>Incidental Inhalation- Powders</i>	NR	NR	6	0.0002**-.06**	NR	NR
<i>Dermal Contact</i>	NR	NR	58	0.000003-0.06	NR	1.2
<i>Deodorant (underarm)</i>	NR	NR	NR	NR	NR	NR
<i>Hair - Non-Coloring</i>	NR	0.00046	186	0.000003-0.31	NR	NR
<i>Hair-Coloring</i>	NR	0.000013	26	NR	NR	NR
<i>Nail</i>	NR	NR	NR	NR	NR	NR
<i>Mucous Membrane</i>	NR	NR	47	0.000003-0.003	NR	NR
<i>Baby Products</i>	NR	NR	NR	NR	NR	NR
	Hydroxypropyl Cyclodextrin		Hydroxypropyltrimonium Hydrolyzed Corn Starch		Hydroxypropyltrimonium Hydrolyzed Wheat Starch	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. Range	53	0.00001-2	11	0.19-0.65	8	NR
Duration of Use						
<i>Leave-On</i>	52	0.00001-2	3	0.24-0.65	NR	NR
<i>Rinse off</i>	1	0.02-0.1	8	0.19-0.43	8	NR
<i>Diluted for (bath) Use</i>	NR	NR	NR	NR	NR	NR
Exposure Type						
<i>Eye Area</i>	13	0.02-1.3	NR	0.65	NR	NR
<i>Incidental Ingestion</i>	NR	0.75	NR	NR	NR	NR
<i>Incidental Inhalation- Sprays</i>	33	0.34-1	3	0.24*	NR	NR
<i>Incidental Inhalation- Powders</i>	29	0.1-2	NR	NR	NR	NR
<i>Dermal Contact</i>	50	0.00001-2	NR	0.65	8	NR
<i>Deodorant (underarm)</i>	1	0.34-2	NR	NR	NR	NR
<i>Hair - Non-Coloring</i>	2	1	11	0.19-0.43	NR	NR
<i>Hair-Coloring</i>	NR	NR	NR	NR	NR	NR
<i>Nail</i>	NR	0.02	NR	NR	NR	NR
<i>Mucous Membrane</i>	NR	0.75	NR	NR	8	NR
<i>Baby Products</i>	NR	NR	NR	NR	NR	NR
	Hydroxypropyl Starch		Hydroxypropyltrimonium Maltodextrin Crosspolymer		Inulin	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. Range	9	0.25-8.2	NR	0.00045	41	0.0005-3
Duration of Use						
<i>Leave-On</i>	8	0.25-8.2	NR	0.00045	14	0.0005-3
<i>Rinse off</i>	1	0.5-6	NR	NR	27	0.25
<i>Diluted for (bath) Use</i>	NR	NR	NR	NR	NR	NR
Exposure Type						
<i>Eye Area</i>	1	NR	NR	NR	1	0.0005
<i>Incidental Ingestion</i>	NR	NR	NR	NR	NR	NR
<i>Incidental Inhalation- Sprays</i>	6	0.25-0.88	NR	NR	8	NR
<i>Incidental Inhalation- Powders</i>	NR	8.2**	NR	NR	9	0.0008**-.2.5**
<i>Dermal Contact</i>	3	0.5-8.2	NR	0.00045	22	0.0005-3
<i>Deodorant (underarm)</i>	NR	NR	NR	NR	NR	NR
<i>Hair - Non-Coloring</i>	6	0.25-1.4	NR	NR	18	NR
<i>Hair-Coloring</i>	NR	NR	NR	NR	NR	NR
<i>Nail</i>	NR	NR	NR	NR	NR	NR
<i>Mucous Membrane</i>	NR	0.5	NR	NR	4	0.25
<i>Baby Products</i>	NR	NR	NR	NR	1	NR

Table 6. Current Frequency and Concentration of Use According to Duration and Type of Exposure.^{16,17,18}

	Laurdimonium Hydroxypropyl Hydrolyzed Wheat Starch		Mannan		Methyl Cyclodextrin	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. Range	6	0.017	19	0.01-0.25	20	4-5
Duration of Use						
<i>Leave-On</i>	NR	NR	16	0.01-0.25	20	4-5
<i>Rinse off</i>	6	0.017	3	NR	NR	NR
<i>Diluted for (bath) Use</i>	NR	NR	NR	NR	NR	NR
Exposure Type						
<i>Eye Area</i>	NR	NR	NR	NR	NR	NR
<i>Incidental Ingestion</i>	NR	NR	NR	NR	NR	NR
<i>Incidental Inhalation- Sprays</i>	NR	NR	11	NR	10	5
<i>Incidental Inhalation- Powders</i>	NR	NR	11	0.01**	NR	NR
<i>Dermal Contact</i>	6	0.017	17	0.01-0.25	19	4-5
<i>Deodorant (underarm)</i>	NR	NR	NR	NR	3	NR
<i>Hair - Non-Coloring</i>	NR	NR	2	NR	1	NR
<i>Hair-Coloring</i>	NR	NR	NR	NR	NR	NR
<i>Nail</i>	NR	NR	NR	NR	NR	NR
<i>Mucous Membrane</i>	6	0.017	NR	NR	NR	NR
<i>Baby Products</i>	NR	NR	NR	NR	NR	NR
	Pectin		Polianthes Tuberosa Polysaccharide		Potassium Alginate	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. Range	87	0.0001-9	2	0.001-0.1	37	1
Duration of Use						
<i>Leave-On</i>	33	0.001-0.05	2	0.001-1	1	1
<i>Rinse off</i>	54	0.0001-9	NR	NR	36	NR
<i>Diluted for (bath) Use</i>	NR	NR	NR	NR	NR	NR
Exposure Type						
<i>Eye Area</i>	4	NR	NR	NR	NR	NR
<i>Incidental Ingestion</i>	NR	0.09-9	NR	NR	NR	NR
<i>Incidental Inhalation- Sprays</i>	25	0.05	2	0.001-0.1*	1	NR
<i>Incidental Inhalation- Powders</i>	17	NR	2	0.001-0.05**	1	NR
<i>Dermal Contact</i>	57	0.05	2	0.001-0.1	37	1
<i>Deodorant (underarm)</i>	NR	NR	NR	NR	NR	NR
<i>Hair - Non-Coloring</i>	30	0.0001-0.05	NR	NR	NR	NR
<i>Hair-Coloring</i>	NR	NR	NR	NR	NR	NR
<i>Nail</i>	NR	NR	NR	NR	NR	NR
<i>Mucous Membrane</i>	1	0.09-9	NR	NR	NR	NR
<i>Baby Products</i>	1	NR	NR	NR	NR	NR
	Potato Starch Modified		Propylene Glycol Alginate		Pueraria Lobata Starch	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. Range	61	0.3-1.3	16	0.00001-0.15	NR	3.6
Duration of Use						
<i>Leave-On</i>	40	0.3-1.3	16	0.00001-0.15	NR	NR
<i>Rinse off</i>	21	1.3	NR	NR	NR	3.6
<i>Diluted for (bath) Use</i>	NR	NR	NR	NR	NR	NR
Exposure Type						
<i>Eye Area</i>	NR	NR	2	NR	NR	NR
<i>Incidental Ingestion</i>	NR	NR	NR	NR	NR	NR
<i>Incidental Inhalation- Sprays</i>	9	1.3*	9	0.005-0.03*	NR	NR
<i>Incidental Inhalation- Powders</i>	5	0.3**	9	0.00001**-0.15**	NR	NR
<i>Dermal Contact</i>	11	0.3-1.3	15	0.00001-0.15	NR	NR
<i>Deodorant (underarm)</i>	NR	NR	NR	NR	NR	NR
<i>Hair - Non-Coloring</i>	49	1.3	1	0.005-0.03	NR	NR
<i>Hair-Coloring</i>	1	NR	NR	NR	NR	3.6
<i>Nail</i>	NR	NR	NR	NR	NR	NR
<i>Mucous Membrane</i>	NR	NR	NR	NR	NR	NR
<i>Baby Products</i>	NR	NR	NR	NR	NR	NR

Table 6. Current Frequency and Concentration of Use According to Duration and Type of Exposure.^{16,17,18}

	Sodium Carboxymethyl Starch		Sodium Carrageenan		Sodium Hydrolyzed Potato Starch Dodecenylsuccinate	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. Range	11	0.05-4.7	3	NR	2	NR
Duration of Use						
<i>Leave-On</i>	3	1.9-4.7	1	NR	NR	NR
<i>Rinse off</i>	8	0.05-2.5	2	NR	2	NR
<i>Diluted for (bath) Use</i>	NR	NR	NR	NR	NR	NR
Exposure Type						
<i>Eye Area</i>	1	4.7	NR	NR	NR	NR
<i>Incidental Ingestion</i>	NR	NR	2	NR	NR	NR
<i>Incidental Inhalation- Sprays</i>	NR	NR	1	NR	NR	NR
<i>Incidental Inhalation- Powders</i>	NR	NR	1	NR	NR	NR
<i>Dermal Contact</i>	2	0.05-4.7	1	NR	NR	NR
<i>Deodorant (underarm)</i>	NR	NR	NR	NR	NR	NR
<i>Hair - Non-Coloring</i>	1	1.9	NR	NR	2	NR
<i>Hair-Coloring</i>	8	2.5	NR	NR	NR	NR
<i>Nail</i>	NR	NR	NR	NR	NR	NR
<i>Mucous Membrane</i>	NR	NR	2	NR	NR	NR
<i>Baby Products</i>	NR	NR	NR	NR	NR	NR
	Sodium Oxidized Starch Acetate/Succinate		Sodium Starch Octenylsuccinate		Solanum Tuberosum (Potato Starch)	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. Range	7	0.05	35	0.0001-0.26	4	3.4-3.6
Duration of Use						
<i>Leave-On</i>	1	0.05	22	0.0001-0.26	2	NR
<i>Rinse off</i>	5	NR	13	0.0023-0.026	2	3.4-3.6
<i>Diluted for (bath) Use</i>	1	NR	NR	NR	NR	NR
Exposure Type						
<i>Eye Area</i>	NR	NR	1	NR	NR	NR
<i>Incidental Ingestion</i>	NR	NR	NR	0.026	NR	NR
<i>Incidental Inhalation- Sprays</i>	1	0.05	16	0.048-0.05	1	NR
<i>Incidental Inhalation- Powders</i>	1	NR	15	NR	1	NR
<i>Dermal Contact</i>	3	NR	21	0.048-0.26	3	NR
<i>Deodorant (underarm)</i>	NR	0.05	4	0.048	NR	NR
<i>Hair - Non-Coloring</i>	4	NR	12	0.0001-0.05	1	3.4
<i>Hair-Coloring</i>	NR	NR	1	NR	NR	3.6
<i>Nail</i>	NR	NR	NR	NR	NR	NR
<i>Mucous Membrane</i>	2	NR	1	0.026	NR	NR
<i>Baby Products</i>	NR	NR	NR	NR	NR	NR
	Starch Acetate		Starch Diethylaminoethyl Ether		Starch Hydroxypropyltrimonium Chloride	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. Range	11	2	1	NR	18	0.002-1.2
Duration of Use						
<i>Leave-On</i>	1	NR	NR	NR	1	0.02-1.2
<i>Rinse off</i>	10	2	1	NR	17	0.002-0.39
<i>Diluted for (bath) Use</i>	NR	NR	NR	NR	NR	NR
Exposure Type						
<i>Eye Area</i>	NR	NR	NR	NR	NR	NR
<i>Incidental Ingestion</i>	NR	NR	NR	NR	NR	NR
<i>Incidental Inhalation- Sprays</i>	NR	NR	NR	NR	1	0.05-1.2*
<i>Incidental Inhalation- Powders</i>	NR	NR	NR	NR	NR	0.02**
<i>Dermal Contact</i>	NR	NR	1	NR	2	0.02
<i>Deodorant (underarm)</i>	NR	NR	NR	NR	NR	NR
<i>Hair - Non-Coloring</i>	11	2	NR	NR	16	0.002-1.2
<i>Hair-Coloring</i>	NR	NR	NR	NR	NR	NR
<i>Nail</i>	NR	NR	NR	NR	NR	NR
<i>Mucous Membrane</i>	NR	NR	1	NR	2	NR
<i>Baby Products</i>	NR	NR	NR	NR	2	NR

Table 6. Current Frequency and Concentration of Use According to Duration and Type of Exposure.^{16,17,18}

	Stearoyl Inulin		Sterculia Urens Gum		Tamarindus Indica Seed Gum	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. Range	9	0.44-4.8	NR	0.2-0.7	NR	0.01-0.3
Duration of Use						
<i>Leave-On</i>	9	0.44-4.8	NR	0.2-0.7	NR	0.05-0.3
<i>Rinse off</i>	NR	NR	NR	NR	NR	0.01-0.25
<i>Diluted for (bath) Use</i>	NR	NR	NR	NR	NR	NR
Exposure Type						
<i>Eye Area</i>	7	0.44-4.8	NR	NR	NR	NR
<i>Incidental Ingestion</i>	NR	NR	NR	NR	NR	NR
<i>Incidental Inhalation- Sprays</i>	NR	NR	NR	NR	NR	NR
<i>Incidental Inhalation- Powders</i>	NR	NR	NR	NR	NR	0.3**
<i>Dermal Contact</i>	9	0.44-4.8	NR	0.7	NR	0.01-0.3
<i>Deodorant (underarm)</i>	NR	NR	NR	NR	NR	NR
<i>Hair - Non-Coloring</i>	NR	NR	NR	NR	NR	0.25
<i>Hair-Coloring</i>	NR	NR	NR	NR	NR	NR
<i>Nail</i>	NR	NR	NR	0.2	NR	NR
<i>Mucous Membrane</i>	NR	NR	NR	NR	NR	NR
<i>Baby Products</i>	NR	NR	NR	NR	NR	NR
	Tapioca Starch		Triticum Vulgare (Wheat) Starch			
	# of Uses	Conc. (%)	# of Uses	Conc. (%)		
Totals/Conc. Range	154	0.45-33	27	0.01-6		
Duration of Use						
<i>Leave-On</i>	124	0.5-33	17	0.01-6		
<i>Rinse off</i>	28	0.45-15	9	0.03-3.6		
<i>Diluted for (bath) Use</i>	2	0.86-32	1	NR		
Exposure Type						
<i>Eye Area</i>	13	NR	5	NR		
<i>Incidental Ingestion</i>	NR	NR	2	0.01		
<i>Incidental Inhalation- Sprays</i>	76	1-15*	1	NR		
<i>Incidental Inhalation- Powders</i>	84	3.7-33	9	NR		
<i>Dermal Contact</i>	115	0.5-33	24	0.03-6		
<i>Deodorant (underarm)</i>	NR	NR	NR	NR		
<i>Hair - Non-Coloring</i>	18	0.45-15	1	NR		
<i>Hair-Coloring</i>	8	3.6	NR	3.6		
<i>Nail</i>	NR	NR	NR	NR		
<i>Mucous Membrane</i>	4	0.86-32	6	0.01		
<i>Baby Products</i>	1	NR	NR	NR		

NR = Not Reported; Totals = Rinse-off + Leave-on + Diluted for (Bath)Use Product Uses.

*It is possible that these products may be sprays, but it is not specified whether the reported uses are sprays.

**It is possible that these products may be powders, but it is not specified whether the reported uses are powders.

***Not specified whether a powder or a spray, so this information is captured for both categories of incidental inhalation

Note: Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure type uses may not equal the sum total uses.

Table 7. Acute Toxicity Studies on Polysaccharide Gums

<i>Inhalation</i>	
<u>Branched - Unmodified</u>	
Glucomannan (in konjac flour): An acute inhalation toxicity study on glucomannan was performed using male and female rats (number and strain not stated). An LC50 of > 0.0015 mg/l was reported. ¹⁵¹	
<i>Oral</i>	
<u>Branched - Unmodified</u>	
Glucomannan: Male and female mice (number and strain not stated). LD ₅₀ > 2,800 mg/kg body weight. No abnormalities with respect to the following: appearance, behavior, body weight changes, occult blood in the urine and feces, or macroscopic findings. ¹⁵²	
Glucomannan (in konjac flour): Male and female rats (number and strain not stated). LD ₅₀ > 5,000 mg/kg body weight. ¹⁵¹	
Sterculia Urens Gum: Vehicle: corn oil. 5 fasted male Sprague-Dawley rats. LD ₅₀ > 10,000 mg/kg body weight. Transient depression, but no other toxic effects. ¹⁵³	
<u>Branched - Modified</u>	
Calcium Starch Isododecenylsuccinate: Material structurally similar to this gum tested. 5 male and 5 female Wistar albino rats. OECD Guideline 401 test protocol. Dosing followed by 14-day observation period. No abnormal systemic signs. LD ₅₀ > 5,000 mg/kg body weight. ^{63,64,154}	
Corn Starch Modified: Vehicle: distilled water. 5 male and 5 female Wistar albino rats. Organisation for Economic Co-operation and Development (OECD) 401 protocol. 14-day observation period. Alopecia in one animal. LD ₅₀ > 2,000 mg/kg body weight. ⁶⁶	
Dextrin Myristate: Rats (number and strain not stated). LD ₅₀ > 2,000 mg/kg body weight. ⁶⁷	
Dextrin Palmitate: Rats (number and strain not stated). LD ₅₀ > 2,000 mg/kg body weight. ^{68,69}	
Potato Starch Modified: 30% aqueous solution. Albino rats (5 males, 5 females). OECD 401 protocol. 14-day observation period. Soft stool (1 female); and no other signs. Body weight changes at necropsy normal. LD ₅₀ > 5,000 mg/kg body weight. ^{70,155}	
Stearoyl Inulin: Rats (number and strain not stated). Protocol not stated. LD ₅₀ > 2,000 mg/kg body weight. ^{71,72}	
<i>Dermal</i>	
<u>Branched - Unmodified</u>	
Glucomannan (in konjac flour): Male and female rabbits (number and strain not stated). Protocol not stated. LD ₅₀ > 2,000 mg/kg body weight. ¹⁵¹	
<u>Branched - Modified</u>	
Carboxymethyl Inulin: 31.1% aqueous carboxymethyl inulin. 10 adult Dunkin–Hartley albino guinea pigs (4 weeks old). Maximization test. No mortality occurred and no clinical signs of systemic toxicity. Body weights and weight gains similar in treated and control groups. ¹⁵⁶	
Corn Starch Modified: Corn starch modified (Amaze® [28-1890]) in distilled water (30% solids). 5 male and 5 female New Zealand White rabbits. OECD 402 protocol. 14-day observation period. Nine of 10 rabbits survived. LD ₅₀ > 2,000 mg/kg body weight. ⁶⁶	
Dextrin Myristate: Rats (number and strain not stated). Occlusive dressing technique (details not included). LD ₅₀ > 2,000 mg/kg body weight. ⁶⁷	
Dextrin Palmitate: Rats (number and strain not stated). Occlusive dressing technique (details not included). LD ₅₀ > 2,000 mg/kg body weight. ^{68,69}	
Potato Starch Modified: 10 rats (strain not specified). OECD 402 test guideline. LD ₅₀ > 2,000 mg/kg body weight. ¹⁵⁵	
Potato Starch Modified: 18.5% solids aqueous solution. 10 New Zealand White rabbits (5 males and 5 females). Semi-occlusive patch application. Dose per cm ² was not stated. Very slight to slight erythema/edema at application sites (all animals); reactions had cleared by 72 h. Signs of local irritation may have been due to mechanical trauma. LD ₅₀ > 2,000 mg/kg body weight. ⁷⁰	
<i>Intravenous</i>	
<u>Linear Polysaccharides and Their Salts</u>	
Carrageenan and Potassium Carrageenan: ι-carrageenan (one subtype of carrageenan with a specific number and position of sulfate groups on the repeating galactose units) or potassium carrageenan (2 mg in phosphate-buffered saline [PBS]). Groups of 5 female MF1 mice. i.v. injection (lateral tail vein). Controls injected with PBS (0.3 ml). Animals killed at 1 h and 24 h post-injection, and tissues prepared for microscopic examination. Carrageenan persisted for at least 6 months in livers and kidneys. Within 24 h of i.v. injection, damage to liver Küpffer cells and changes in the microcirculation characteristic of disseminated intravascular coagulation (DIC) in the liver and kidney observed. No adverse effects in hepatocytes, but chronic renal damage observed. ι-carrageenan less toxic to liver and kidney, compared to the potassium carrageenan (less pure, compared to ι-carrageenan). ¹⁵⁷	
Carrageenan and Potassium Carrageenan: ι-carrageenan or potassium carrageenan in saline (0.5 ml or 1 ml i.v. injection). Groups of 9 to 15 female CAF ₁ mice (Balb/c x A/He). 7- or 14-day observation period. Treatment with either compound induced anemia, granulocytosis, and early profound thrombocytopenia. Treatment with ι-carrageenan caused an early lymphocytosis, and both compounds induced lymphopenia by 18 h post-treatment. Treatment with either compound was associated with an early moderate reduction in the number of nucleated cells and granulocyte/macrophage colony-forming cells per femur. Each compound induced splenomegaly, and ι-carrageenan-treated mice developed hypoplasia of the thymus by 18 h post-injection. Sustained increase in numbers of colony-forming cells in spleen after treatment with each compound. ¹⁵⁸	

Table 7. Acute Toxicity Studies on Polysaccharide Gums

<i>Intrapleural</i>
Linear Polysaccharides and Their Salts Carrageenan: Groups of 6 adult female Balb/c mice (6 to 7 weeks old). One group received single intrapleural injection of 0.1 ml sterile saline (0.9% NaCl) and λ-carrageenan (one subtype of carrageenan with a specific number and position of sulfate groups on the repeating galactose units; 1% in solvent [not stated]), which induced pleurisy. Another group each received single intrapleural injection of 1% λ-carrageenan (0.1 ml) only. Animals were killed, and lung tissue samples obtained for microscopic examination at 4 h and 24 h post-injection. Dense inflammation with lobar lung pneumonia and thickened alveolar septum (with occasionally obliterated alveoli) were observed.¹⁵⁹ Carrageenan: Injection of 2% λ-carrageenan in saline (200 mg/kg) into pleural cavity. Groups of 10 mice. Dosing caused pleurisy, characterized by marked accumulation of fluid and the migration of leukocytes to the site of inflammation in lung.¹⁶⁰
<i>Transbronchial</i>
Linear Polysaccharides and Their Salts Carrageenan: Transbronchial injection of 0.75% carrageenan in physiological saline. 27 male albino rabbits. Surviving animals were killed according to the following schedule: 2 at 24 h; 3 each at 3 days, 1 and 2 weeks, and 1 month; 5 at 2 months; and 8 at 4 months. Pneumonia, followed by emphysema in the insulted lung, observed. Of the 8 animals injected with carrageenan and killed at 4 months, 3 were deemed inappropriate for morphometry because of developing fibrosis, abscesses and/or emphysematous bullae in the lungs. Thus, the lungs (mild to severe erythema observed) of the remaining 5 animals injected with carrageenan and of the 5 control rabbits killed at 4 months were prepared for morphometric analysis. Scattered infiltration of polymorphonuclear leukocytes throughout the affected lobe, subsequently replaced by accumulation of carrageenan-laden macrophages; changes lasted for 1 to 2 months. Enlargement of alveoli and alveolar ducts observed at 2 weeks to 2 months post-injection, and pulmonary emphysema observed at 4 months. The lobes not injected with carrageenan had normal appearance throughout study.¹⁶¹

Table 8. Repeated Dose Toxicity Studies on Polysaccharide Gums

Oral - Non-Human

Linear Polysaccharides and Their Salts

Algin: 25% Sodium alginate (also known as algin) in diet. Mice (75 males and 75 females). Feeding with sodium alginate in the diet for 89 weeks. At week 87, half of the surviving male and female mice in each test group placed on control diet (containing 55% pregelatinized potato starch). During feeding period, dietary levels of test substances gradually increased until diets contained (by weight) 25% sodium alginate. All survivors killed during weeks 89 to 92. Sodium alginate caused increased water consumption, distinct caecal and colonic enlargement, and a slightly increased incidence of intratubular nephrosis. Sodium alginate was nephrotoxic, causing increased kidney weights, distension of the renal calyx and high incidence of dilated distal tubules.¹⁶²

Carrageenan: 25,000 ppm or 50,000 ppm kappa carrageenan. Groups of Fischer 344 rats (20/sex/group). Feeding in diet for 90 days. Clinical signs limited to soft feces in high dose rats, and to a lesser extent, in low dose rats. No treatment-related effects on body weights, urinalysis, hematology or clinical chemistry parameters, or on organ weights or ophthalmic, macroscopic or microscopic findings. Gastrointestinal tract appeared normal in detailed histopathological evaluation. NOAEL = 50,000 ppm (mean calculated test material consumption of 3394 ± 706 mg/kg/day in males and 3867 ± 647 mg/kg/day in females).¹¹³

Carrageenan: kappa/lambda-carrageenan (from *C. crispus* or *G. mamillosa*) at concentrations of 0, 0.1, 5, 15, or 25%. Five male and five female mice of 2 unidentified strains. Lifetime dietary feeding had no adverse effect. Same test material and dietary concentrations. Five male and 5 female rats of 2 unidentified strains. Lifetime dietary feeding. Evidence of hepatic cirrhosis, only at the 25% concentration, with no effect on mortality.⁶²

Carrageenan: Extracts of kappa-carrageenan (from *Hypnea musciformis* or *Irideae crispata*) at concentration of 1% or 5%. Groups of 15 male and female Sprague-Dawley rats. Feeding in diet for 1 year. Weight loss ($p = 0.05$) in all treatment groups, compared to control (alphacel) group. Livers of rats fed 1% concentration normal at gross and microscopic examination. Livers from rats given 5% kappa-carrageenan from *H. musciformis* normal at gross and microscopic examination, except for nodules in 2 of 12 livers. Gross examination of livers from rats fed 5% kappa-carrageenan (from *I. crispata*) showed decreased size, rough surface, and vascularization in 10/13 rats, probably treatment-related. Microscopically, these livers were normal, except for focal necrosis in 1 of 10 livers. No evidence of storage of carrageenan-like material (metachromatic) in liver cells of any of the treated rats, and no fibrillar material observed using electron microscopy. No changes observed in stools of rats receiving 1% of either carrageenan. Loose stools in female rats given 5% kappa-carrageenan from *I. crispata* and in males given either carrageenan at 5% concentrations. Blood found sporadically in stools, but frequency was not significant.⁶²

Carrageenan: kappa/lambda-carrageenan. Groups of 19 male and 21 female rhesus monkeys. Feeding (gavage) with 0, 50, 200, or 500 mg/kg body weight (6 days/week for five years, and dietary feeding for an additional 2.5 years. Random distribution of loose stools, chronic intestinal disorders, poor appetite, and emaciation. Stool consistency decreased in dose-related trend over entire 7.5 years of the study; findings of fecal occult blood increased in similar fashion. Mean survival time similar in all groups; no gross or microscopic changes in tissues examined. Sporadic differences in body weight observed randomly. Females had significant body-weight depression (not dose-related) in last 2.5 years of study. No consistent, statistically significant changes in hematological or clinical chemical values, absolute organ weights, or organ-to-body weight ratios after 7.5 years of feeding. Cytochemical and ultrastructural observations revealed no storage of carrageenan-like material in livers, obtained at biopsy or in other organs obtained at necropsy; no dose-related gross or microscopic changes in other tissues.⁶²

Inulin: 7.5% inulin. 20 Wistar rats of the CrI:(WI)BR strain (10 males, 10 females). Daily dietary feeding for 13 weeks. No remarkable microscopic or macroscopic findings.¹⁶³

Branched - Unmodified

Arabinoxylan: Wheat bran extract (~ 80% arabinoxylan oligopeptides) at concentrations of 0.3%, 1.5%, and 7.5%. 3 groups of 20 Wistar rats of the CrI:(WI)BR strain (10 males/group, 10 females/group). Feeding resulted in average daily intakes of 0.2 g/kg (0.3% concentration), 0.9 g/kg (1.5%), and 4.4 g/kg (7.5%) for 13 weeks. No evidence of test substance-related adverse macroscopic or microscopic findings. At histopathological examination, minimal bilateral hypertrophy of renal cortical tubules in males and females, particularly in highest-dose group. Findings were not accompanied by degenerative changes or changes in kidney weight, and were considered non-toxic and suggestive of an adaptive response. No remarkable findings in control rats fed basal diet. NOAEL = 4.4 g/kg/day.¹⁶³

Ghatti Gum: Ghatti gum concentrations of 0, 0.5, 1.5 and 5%. Groups of Sprague-Dawley rats (10 males/group, 10 females/group). Dietary feeding (in basal diet) for at least 90 days. Ghatti gum intake at 5% dietary level ranged from 3044 to 3825 mg/kg body weight/day. Feed consumption among treated and control groups was similar for males and females. 2 of 10 females in 5% ghatti gum group had a single colon ulcer, with associated acute inflammation. Ulcers were considered sporadic occurrences, possibly attributable to basal diet. NOAEL = 5% in diet; NOAELs for males and females estimated at 3044 and 3309 mg/kg/day, respectively.¹⁶⁴

Ghatti Gum: 5% Ghatti gum. Groups of 20 female Sprague-Dawley rats. Dietary feeding for at least 90 days. Single colon ulcer, with associated acute inflammation, in 1 of 20 control females given basal diet. Colon ulcer considered sporadic, possibly attributable to basal diet. Statistically significant alterations in clinical chemistry were considered sporadic and unrelated to treatment. Feed consumption among treated and control groups similar for each sex. NOAEL = 5% in diet; NOAELs at 3670 and 3825 mg/kg/day for different control diets.¹⁶⁴

Glucomannan: 10% konjac (plant consisting mostly of glucomannan). Groups of four male Sprague-Dawley rats were fed either 5% cellulose (control), 10% pectin, or 10% konjac for 28 days. After dosing period, rats were fasted for 24 h, fed 5 g/kg body weight brown rice, and killed 5 h later. No indication of toxicity.^{165,166}

Table 8. Repeated Dose Toxicity Studies on Polysaccharide Gums**Branched - Unmodified**

Glucomannan: 2.5%, 5%, or 10% refined konjac meal. Groups of 12 five-week-old Sprague-Dawley rats of each sex. Feeding with either a normal basal diet, a hypercholesterolaemic diet (control diet containing 1% cholesterol), or one of three test diets. Because refined konjac meal contains ~80% glucomannan, the highest concentration of glucomannan tested was ~8%. Four animals of each sex from each group killed after 4, 8, and 12 weeks of feeding. Histological and gross examination of livers from rats fed 1% cholesterol showed spreading fatty degeneration with focal necrosis and a nonspecific inflammation reaction. Similar changes observed in group receiving refined konjac meal at the end of 4 weeks, but the changes disappeared gradually with longer feeding times, and the morphology of the liver was similar to that in the normal control group at the end of 12 weeks. Changes were also observed at gross examination of the liver.¹⁶⁷

Glucomannan: Basal diet in which 1% of the cornstarch replaced with refined glucomannan (i.e., 1% konjac meal). Groups of 15 Sprague-Dawley rats of each sex. Dietary feeding for 18 months. At the end of feeding period, the animals were killed and the brain, liver, aorta, kidney, spleen, and heart removed. At microscopic examination, the livers of treated rats contained smaller, more lightly stained nuclei and reduced bile-duct proliferation in the portal area. Endothelial cells in the aorta of treated animals were smaller and there was less thickening of the aortic wall. These changes were related to less senescence in the treated group than in the control group. No evidence of treatment-related pathological changes. NOAEL = 1% konjac meal, equivalent to an intake of 500 mg/kg body weight per day.¹⁶⁵

Pectin and Solanum Tuberosum (Potato) Starch: Test diets containing 5% or 10% pectin-derived acidic oligosaccharides (pAOS). Two groups of F₁ rats (from outbred strain of Wistar rats (CrI:WI(WU); number not stated). Dietary feeding with test (± 7 g/kg body weight/day) and control diets for 13 weeks. To keep the total level of added test substance equal in each diet, the low-dose diet (5% pAOS) was adjusted with 5% potato starch. One control group received the standard rodent diet supplemented with 10% potato starch, and the other control group received 10% short-chain FOS (scFOS) in the diet. No treatment-related clinical signs observed, and none of the rats died. Ophthalmoscopic examination did not reveal any treatment-related ocular changes. Neurobehavioral examination and motor activity assessment did not indicate any neurotoxic potential. No relevant differences in body weight, growth rate and feed intake. Macroscopic examination at necropsy did not reveal any adverse effects. Microscopic examination revealed treatment-related histopathological changes in the urinary bladder of animals of the 10% pAOS group. One male and one female of the 5% pAOS group and one male of the control group showed diffuse hyperplasia (very slight). In addition, two males and two females of the 5% pAOS group showed simple hyperplasia in a part of the urinary bladder lining ('focal hyperplasia'). No treatment-related hyperplasia of the transitional epithelium was observed in the kidney. Administration of pAOS at dietary levels up to 10% (equivalent to 7.1 g/kg body weight/day) did not reveal any relevant effects that could be attributed to the ingestion of acidic oligosaccharides.¹⁶⁸

Starch Acetate: 55% Starch acetate (a chemically modified potato starch) in diet. Mice (75 males and 75 females per test substance). Feeding with starch acetate in the diet for 89 weeks. At week 87, half of the surviving male and female mice placed on control diet (containing 55% pregelatinized potato starch). During feeding period, dietary level of test substance gradually increased until diet contained (by weight) 55% starch acetate. All survivors killed during weeks 89 to 92. Starch acetate caused increased water consumption, distinct caecal and colonic enlargement, and a slightly increased incidence of intratubular nephrosis. Increased incidence of gastric trichobezoars. Concretions in renal pelvis with slight urinary changes, such as increased amounts of amorphous material in the urine and increased urinary calcium content, in the mice fed starch acetate not toxicologically significant. The incidence of intratubular calcinosis or concretions in the pelvic space was not reduced during the recovery period. Caecal and colonic enlargement and changes in urinalysis results were found to be reversible.¹⁶²

Sterculia Urens Gum: 5 non-fasted male Sprague-Dawley rats. Animals intubated with 5 g/kg/day, daily for 5 days. No adverse effects.¹⁵³

Sterculia Urens Gum: 7% (w/w) sterculia urens gum. Albino Wistar rats (rats housed 3 per cage; number tested not stated) Transmission electron microscopy used to study ultrastructure of jejunum, ileum, and cecum after dietary supplementation for 45 days [15 micrographs analyzed] for 45 days. No abnormalities in any of the organelles.¹⁶⁹

Branched - Modified

Carboxymethyl Inulin: Carboxymethyl inulin (31.1% aqueous). Groups of five male and five female Wistar CrI rats. Doses of 0, 50, 150 and 1000 mg/kg/day (by gavage) for 4 weeks. In all dose groups, no treatment-related effects with respect to: body weight, feed consumption, mortality, hematology, clinical blood chemistry, organ weights or gross or microscopic pathology.¹⁵⁶

Cyclic

Cyclodextrin: β -cyclodextrin (12,500, 25,000 and 50,000 ppm). Groups of 40 (20 males, 20 females/group) CrI:CD (SD) BR Sprague-Dawley rats. Feeding in the diet for 52 weeks. Control group fed basal diet. The liver and kidney were identified at histopathological examination as target organs for toxicity at concentrations of 50,000 ppm and 25,000 ppm, with the hepatic changes associated with increased plasma liver enzyme and decreased plasma triglyceride concentrations. The only finding for kidneys was a statistically significant ($p < 0.01$) increased incidence of minimal/trace amounts of pigment in the epithelium of the cortical tubules in female rats that received 25,000 ppm or 50,000 ppm β -cyclodextrin in the diet. The "non-toxic dietary inclusion level" of β -cyclodextrin was 12,500 ppm (equivalent to 654 or 864 mg/kg/day for males or females, respectively).⁴⁴

Cyclodextrin: β -cyclodextrin (6200, 12,500 and 50,000 ppm). Groups of 8 (4males, 4 females/group) pure-bred Beagle dogs. Preceding test protocol in rat study used. No pathological evidence of systemic toxicity, although there were minor changes in urinalysis and biochemical parameters and a slightly higher incidence of liquid feces. These changes were considered to be of no toxicological importance. The "non-toxic dietary inclusion level" of β -cyclodextrin was 50,000 ppm (equivalent to 1,831 or 1,967 mg/kg/day for males or females, respectively).⁴⁴

Cycodextrin: γ -cyclodextrin (5%, 10%, or 20%). Groups of 8 (4 males, 4 females) Beagle dogs. Feeding in the diet for 13 weeks. Control group fed basal diet. No treatment-related changes in behavior or appearance and no mortalities. No treatment-related differences with respect to ophthalmoscopic examinations, hematological parameters, clinicochemical analyses of the plasma, and semiquantitative urine analyses. Relative ovary weights significantly increased in the 10% and 20% concentration groups, but this observation was probably a result of an unusually low ovarian weight in the controls. An increase in relative liver weights in males of the 10% and 20% concentration groups was also considered to lack toxicological relevance, because this observation was not associated with changes in plasma enzyme levels or with histopathological changes. No treatment-related abnormalities observed at necropsy. At microscopic examination, no treatment-related effects in any of the various organs and tissues. Daily consumption of up to 20% γ -cyclodextrin in the diet (≈ 7.7 g/kg body weight in males and 8.3 g/kg body weight in females) did not cause toxicity.¹⁷⁰

Table 8. Repeated Dose Toxicity Studies on Polysaccharide Gums*Oral - Human***Branched - Unmodified**

Sterculia Urens Gum: 5 male volunteers (30 to 56 years old). Ingestion of sterculia urens gum (10.5 g in diet) daily for 21 days. No toxicity or significant effects on plasma biochemistry, hematological indices, or urinalysis parameters were noted.¹⁷¹

Branched – Modified

Propylene Glycol Alginate: 5 male volunteers. Following a 7-day control period, the men consumed an amount of propylene glycol alginate equal to 175 mg/kg body weight during the first 7 days of the test period. The amount consumed was increased to 200 mg/kg body weight for the remainder (i.e., 16 days) of the 23 days of dietary supplementation. No significant effect (statistical analysis not performed) on the following: hematological indices, plasma biochemistry parameters, urinalysis parameters, blood glucose levels, plasma insulin concentrations, and expired hydrogen concentrations. Ingestion of propylene glycol alginate caused no adverse dietary or physiological effects. The enzymatic indicators of toxicological effects remained unchanged.⁵³

*Dermal - Non-Human***Branched - Modified**

Carboxymethyl Inulin: 31.1% aqueous carboxymethyl inulin. 10 adult Dunkin–Hartley albino guinea pigs. Maximization test. 5 female guinea pigs (vehicle controls). No mortalities or clinical signs of systemic toxicity were observed. Body weights and weight gains were considered similar when treated and control groups were compared.¹⁵⁶

Potato Starch Modified: Rats (10 males, 10 females). Applied to skin under occlusive dressing for 28 days (2 g/kg body weight/day) according to OECD 410 test guideline. Sporadic gains and losses of body weight. Compared to the vehicle control group, statistically significant (p value not stated) decrease in body weight gain in treated females during weeks 1 and 4. Clinical biochemical test results indicated statistically significant (p value not stated) decrease in serum triglycerides and slight increase in serum calcium, sodium, and phosphorus in treated males, but not in females. However, none of the other test parameters supported these findings. Decreased organ weights and differences in hematologic test parameters, but these findings were within historical control ranges for this strain of rat. Signs of systemic toxicity not observed at gross examination of treated animals. NOAEL $\geq$ 2,000 mg/kg body weight/day.¹⁵⁵

Potato Starch Modified: 10% solids aqueous solution. New Zealand albino rabbits (10 males and 10 females) tested; 20 rabbits (controls). Applied to skin under a non-occlusive patch (dose = 2 g/kg bodyweight). Area of application and concentration/dose per cm² were not stated. Distilled water, under a non-occlusive patch, applied to controls. Daily evaluations for signs of systemic toxicity, mortality, or morbidity occurred daily; necropsy on day 28. The following considered within normal parameters: body weights, food consumption, gross pathology, and histopathology. Minor differences in organ weight and clinical chemistry changes observed, but considered irrelevant. No significant toxic effects in rabbits.⁷⁰

Table 9. Reproductive and Developmental Toxicity Studies on Polysaccharide Gums

Ingredient	Animals	Procedure	Results
<i>Linear polysaccharides and their salts</i>			
Ammonium Alginate	Fertile eggs from Single-comb White Leghorn chickens	Single injection of ammonium alginate (in corn oil, $\leq 100 \mu\text{l}$) into groups of 20 or more eggs; doses up to 0.5 mg/egg)	Injection did not result in significant numbers of abnormal birds. ¹⁷²
<i>kappa/lambda</i> -Carrageenan (from <i>C. crispus</i>) sodium or calcium salt	Groups of 22 to 27 pregnant CD-1 mice	Oral doses of 10, 45, 470, or 900 mg/kg body weight/day on days 6-15 of gestation	Number of fetal resorptions and/or fetal deaths increased. Dose-dependent decrease in number of live pups and pup weight. Skeletal maturation was retarded. A no-observed-effect level was not reported. ⁶²
<i>kappa/lambda</i> -Carrageenan (from <i>C. crispus</i>) sodium or calcium salt	Groups of 21 to 27 pregnant rats (strain not stated)	Oral doses of 40, 100, 240, or 600 mg/kg body weight/day on days 6-15 of gestation	Increased fetal resorptions, with no decrease in the number of live pups. Dose-dependent increase in incidence of missing skeletal sternebrae. ⁶²
<i>kappa/lambda</i> -Carrageenan (from <i>C. crispus</i>) sodium or calcium salt	Groups of 21 to 24 pregnant rats (strain not stated)	Feeding with 1% or 5% in diet on days 6-16 of gestation	Neither salt was teratogenic. ⁶²
<i>kappa/lambda</i> -Carrageenan (from <i>C. crispus</i>) calcium salt	40 male and 40 female Osborne-Mendel rats	Three-generation study. Feeding with 0.5, 1, 2.5, or 5% in diet 12 weeks prior to mating	In F _{2c} and F _{3c} litters, no specific external, skeletal, or soft-tissue anomaly could be correlated with dosage. ⁶²
Calcium Carrageenan	Sprague-Dawley rats (number not stated)	Feeding with 0.45, 0.9, or 1.8% in diet prior to mating, during breeding, and throughout gestation, lactation, and post-weaning	No differences between test and negative control groups regarding length of gestation, litter size, or sex distribution. ^{62,173}
<i>kappa/lambda</i> -Carrageenan (from <i>C. crispus</i>) sodium or calcium salt	Groups of 23 to 30 pregnant hamsters (strain not stated)	Oral doses of 40, 100, 240, or 600 mg/kg body weight on days 6-10 of gestation	No significant effect on nidation or on maternal or fetal survival. Some evidence of dose-dependent delay in skeletal maturation. ⁶²
<i>kappa/lambda</i> -Carrageenan (from <i>C. crispus</i>) sodium or calcium salt	Groups of 21 to 26 pregnant hamsters	Feeding with 1% or 5% in diet on days 6-11 of gestation	Neither salt was teratogenic. ⁶²
Carrageenan (sodium or calcium salt) or degraded Carrageenan	21 pregnant female Syrian hamsters per dose of carrageenan; 8 pregnant females per dose of degraded carrageenan	Oral doses of 10, 40, 100, or 200 mg/kg body weight on days 6-10 of gestation	No dose-related teratogenic or fetotoxic effects. ⁶²
<i>kappa/lambda</i> -Carrageenan (from <i>C. crispus</i>) sodium or calcium salt	Groups of 12 to 13 pregnant female rabbits (strain not stated)	Oral doses of 40, 100, 240, or 600 mg/kg body weight on days 6-18 of gestation	The numbers of skeletal or soft tissue abnormalities did not differ from those of controls. ⁶²

Table 9. Reproductive and Developmental Toxicity Studies on Polysaccharide Gums

Ingredient	Animals	Procedure	Results
<i>Branched - unmodified</i>			
Glucomannan (from <i>Amorphophallus oncophyllus</i>)	6 pregnant British short-hair domestic cats	Concentration of 2% in the diet during gestation. Actual intake during week prior to parturition ranged from 0.98 to 3.08 mg/kg body weight per day	All pregnant females completed lactation and a normal gestation period. No adverse effect on mean birth weight or mean litter size. ¹⁰⁵
Pectin-derived acidic oligosaccharides (pAOS)	Groups of 24 (16 females, 8 males per group) parental (F ₀) Wistar rats of the cri:WI(WU) outbred strain	Concentrations of 5% or 10% in the diet prior to mating, and throughout mating, gestation, and lactation periods	No effect on estral cycle length and normality. No relevant changes in sperm motility, sperm count, or morphologic changes. No effects on reproductive indices, including litter size, pup viability, and difference in sex ratio. ¹⁶⁸
Sterculia Urens Gum (suspension in anhydrous corn oil)	Groups of 87 to 90 pregnant female Dutch-belted rabbits	Oral doses up to 635 mg/kg/day for 13 consecutive days (gestation days 8-18).	Not teratogenic. ¹⁷⁴
Sterculia Urens Gum (suspension in anhydrous corn oil)	Groups of 87 to 90 pregnant female albino CD-1 mice	Oral doses up to 170 mg/kg body weight on days 6 through 15 of gestation	No clearly discernible effect on nidation or on maternal or fetal survival. No difference in soft or skeletal tissue abnormalities between test animals and sham-treated controls. Not teratogenic. ¹⁷⁴
Sterculia Urens Gum (suspension in anhydrous corn oil)	28 pregnant female albino CD-1 mice	Oral dose of 800 mg/kg body weight on days 6 through 15 of gestation	Significant number of maternal deaths (9 of 28). Surviving dams were completely normal and delivered normal fetuses, with no effect on rate of nidation, or live pup survival <i>in utero</i> . Not teratogenic. ¹⁷⁴
Sterculia Urens Gum (suspension in anhydrous corn oil)	Groups of 87 to 89 pregnant female Wistar-derived albino rats	Oral doses up to 900 mg/kg body weight on days 6 through 15 of gestation	Dams were completely normal and delivered normal fetuses, with no effect on rate of nidation, or live pup survival <i>in utero</i> . Not teratogenic. ¹⁷⁴
<i>Branched - modified (i.e., added sidechains are larger than acetate)</i>			
Propylene Glycol Alginate	Fertile eggs from Single-comb White Leghorn chickens	Single injection of propylene glycol alginate (in water, ≤ 100 µl) into groups of 20 or more eggs; doses up to 1 mg/egg)	Injection did not result in significant numbers of abnormal birds. ¹⁷²
<i>Cyclic</i>			
γ-Cyclodextrin	Groups of 25 pregnant female Wistar Cri (WI)WU BR rats	Concentrations of 1.5%, 5%, 10%, and 20% in the diet on gestation days 0 to 21.	No fetotoxic embryotoxic, or teratogenic effects. NOAEC ≈ 20% in diet (≈ 11 g/kg body weight per day). ¹⁷⁵
α-Cyclodextrin	Groups of 25 pregnant female Wistar Cri (WI)WU BR rats	Concentrations of 1.5%, 5%, 10%, and 20% in the diet on gestation days 0 to 21.	No fetotoxic embryotoxic, or teratogenic effects. NOAEC = 20% in diet (≈ 13 g/kg body weight per day). ¹⁷⁶
γ-Cyclodextrin	Groups of 16 pregnant female New Zealand White rabbits	Concentrations of 5%, 10%, or 20% in the diet on gestation days 0 to 29.	No effect on reproductive performance, and not fetotoxic, embryotoxic, or teratogenic. ¹⁷⁷
α-Cyclodextrin	Groups of 16 pregnant female New Zealand White rabbits	Concentrations of 5%, 10%, or 20% in the diet on gestation days 0 to 29.	No effect on reproductive performance, and not fetotoxic, embryotoxic, or teratogenic. ¹⁷⁸

Table 10. Genotoxicity of Polysaccharide Gums

Ingredient/Similar Chemical	Strain/cell type	Assay	Dose	Results
<i>Bacterial Assays</i>				
<i>Linear polysaccharides and their salts</i>				
Carrageenan (natural grade [PNG]) or refined Carrageenan	<i>Salmonella typhimurium</i> strain TA100	Ames test	Concentrations up to 100 mg/ml (PNG) and up to 25 mg/ml (refined) without metabolic activation	Not genotoxic. ¹⁷⁹
<i>kappa/lambda</i> -Carrageenan (from <i>C. crispus</i>)	<i>Salmonella typhimurium</i> strains TA1535, TA1537, and TA1538. <i>Saccharomyces cerevisiae</i> strain D4.	Ames test	Test concentrations not stated	Not genotoxic. ⁶²
PNG or Refined Carrageenan	Mice (strain not stated). <i>Salmonella typhimurium</i> strain His G 46	Host-mediated assay	Mice received PNG at oral doses up to 2,500 mg/kg body weight or refined carrageenan at a dose of 700 mg/kg body weight. Bacterial strain tested without metabolic activation	Mutation frequency in injected indicator organism not affected by dosing with carrageenan. Neither PNG nor refined carrageenan was genotoxic. ¹⁷⁹
PNG or Refined Carrageenan	<i>Bacillus subtilis</i>	Rec assay for DNA-damaging potential	PNG and refined carrageenan tested at concentrations up to 100 mg/ml and 28 mg/ml, respectively	Neither PNG nor refined carrageenan was genotoxic. ¹⁷⁹
<i>Linear - modified</i>				
Hydrolyzed furcellaran trade name mixture (0.6% hydrolyzed furcellaran, 0.05% concentrate of sea water, 1% phenoxyethanol, and 98.35% water)	<i>Salmonella typhimurium</i> strains TA97a, TA98, TA100, and TA 1535; <i>E. coli</i> strain WP2uvrA pKM101	Ames test	Doses and presence/absence of activation not stated	Not genotoxic. ⁷³
<i>Branched - unmodified</i>				
Arabinoxylan	<i>Salmonella typhimurium</i> strains TA98, TA 100, TA 1535, and TA 1537; <i>Escherichia coli</i> (<i>E. coli</i>) strain WP2uvrA	Ames test	up to 5,000 µg/plate, with and without metabolic activation	Not genotoxic. ¹⁶³
Ghatti gum	<i>Salmonella typhimurium</i> strains TA97a, TA98, TA100, and TA 1535; <i>E. coli</i> strain WP2uvrA pKM101	Ames test	6 mg/plate, with and without metabolic activation	Not genotoxic. ¹⁸⁰
Glucomannan (in konjac flour)	<i>Salmonella typhimurium</i> (5 strains, not stated)	Ames test	With and without metabolic activation (doses not stated)	Not genotoxic. ¹⁵¹
Pectin-derived acidic oligosaccharides (mixture of linear oligomers and small polymers of galacturonic acid) (for genotoxicity evaluation of Pectin)	<i>Salmonella typhimurium</i> strains TA98, TA 100, TA 1535, and TA 1537; <i>E. coli</i> strain WP2uvrA	Ames test	up to 5,000 µg/plate, with and without metabolic activation	Not genotoxic. ¹⁶⁸

Table 10. Genotoxicity of Polysaccharide Gums

Ingredient/Similar Chemical	Strain/cell type	Assay	Dose	Results
Sterculia urens gum	Mice (strain not stated). <i>Salmonella typhimurium</i> strains G46 and TA1530 and <i>Saccharomyces cerevisiae</i> strain D3	Host-mediated assay	3 groups of mice intubated with 5,000 mg/kg, 2500 mg/kg, and 30 mg/kg, respectively, followed by injection with tester strains	Not genotoxic in plated tester strains. ¹⁵³
<i>Branched - modified (i.e., added sidechains are larger than acetate)</i>				
Carboxymethyl inulin	<i>Salmonella typhimurium</i> strains TA98, TA 100, TA 1535, and TA 1537; <i>Escherichia coli</i> (<i>E. coli</i>) strain WP2uvrA	Ames test	Same as above	Not genotoxic. ¹⁵⁶
Calcium Starch Isododecenylsuccinate	<i>Salmonella typhimurium</i> strains TA98, TA 100, TA 1535, and TA 1537; <i>E. coli</i> strain WP2uvrA	Ames test	up to 5,000 µg/plate, with and without metabolic activation	Not genotoxic. ⁶³
Corn starch modified (Amaze® [28-1890])	<i>Salmonella typhimurium</i> strains TA98, TA 100, TA 1535, or TA 1537; <i>E. coli</i> strain WP2uvrA	Ames test	up to 5,000 µg/plate, with and without metabolic activation	Not genotoxic. ⁶⁶
Dextrin myristate (Rheoparl MKL2)	<i>Salmonella typhimurium</i> (strains not stated)	Ames test	Doses and presence/absence of activation not stated	Not genotoxic. ⁶⁷
Dextrin palmitate (Rheoparl KL2 and Rheoparl TL2)	<i>Salmonella typhimurium</i> (strains not stated)	Ames test	Doses and presence/absence of activation not stated	Not genotoxic. ^{68,69}
Dextrin isostearate (Unifilma HVY)	<i>Salmonella typhimurium</i> and <i>E. coli</i> (strains not stated)	Ames test	Doses and presence/absence of activation not stated	Not genotoxic. ¹²⁷
Sodium Hydrolyzed Potato Starch Dodecenylsuccinate trade name material (PS-111 hydrophobically modified starch powder)	<i>Salmonella typhimurium</i> strains TA98, TA 100, TA 1535, and TA 1537; <i>E. coli</i> strain WP2uvrA	Ames test	up to 5,000 µg/plate, with and without metabolic activation	Not genotoxic. ¹⁸¹
Stearoyl inulin (Rheoparl ISK2 and Rheoparl ISL2)	<i>Salmonella typhimurium</i> and <i>E. coli</i> (strains not stated)	Ames test	Doses and presence/absence of activation not stated	Not genotoxic. ^{71,72}
<i>Mammalian Assays</i>				
<i>Linear polysaccharides and their salts</i>				
PNG or Refined Carrageenan	Bone marrow cells from Swiss mice	Micronucleus test	Mice received PNG at doses up to 2,500 mg/kg body weight or refined carrageenan at a dose of 700 mg/kg body weight	Neither PNG nor refined carrageenan was genotoxic. ¹⁷⁹
<i>Branched - modified (i.e., added sidechains are larger than acetate)</i>				
Carboxymethyl inulin	Chinese hamster ovary (CHO-WBL) cells	Chromosome aberrations assay	up to 5,060 µg/ml, with and without metabolic activation	No significant increases in chromosomal aberrations, polyploidy, and endoreduplication. ¹⁵⁶

Table 10. Genotoxicity of Polysaccharide Gums

Ingredient/Similar Chemical	Strain/cell type	Assay	Dose	Results
Potato starch modified	Mice (strain not stated)	Mouse lymphoma assay. OECD 476 test guideline.	Not stated	Not genotoxic. ¹⁵⁵
<i>Branched - unmodified</i>				
Ghatti gum	Chinese hamster ovary (CHO-WBL) cells	Chromosome aberrations assay	up to 6,000 µg/ml, with and without metabolic activation	Not genotoxic. ¹⁸⁰
Ghatti gum	B6C3F1 mice	Combined micronucleus/Comet assay	Mice dosed orally with up to 2,000 mg/kg/day for 4 days	No effect on micronucleated reticulocyte frequency in peripheral blood. No DNA damage in blood leukocytes or liver. ¹⁸⁰
Glucomannan	L5178Y tk ^{+/+} mouse lymphoma cells	Mouse lymphoma assay	Up to 1,000 µg/ml and up to 997 µg/ml with and without metabolic activation, respectively	Not genotoxic. ¹⁶⁵
Glucomannan	CD-1 (ICR) mouse bone marrow cells	Micronucleus test	Mice dosed orally with 5,000 mg/kg body weight	Not genotoxic. ¹⁶⁵
Pectin-derived acidic oligosaccharides (for genotoxicity evaluation of Pectin)	L5178Y mouse lymphoma cells	Mouse lymphoma assay	up to 4370 µg/ml, with and without metabolic activation	Equivocal results. ¹⁶⁸
Pectin-derived acidic oligosaccharides (for genotoxicity evaluation of Pectin)	Chinese hamster ovary cells	Chromosome aberrations assay	up to 4,220 µg/ml, with and without metabolic activation	Clastogenic. Dose-related genotoxicity at ≥ 2,530 µg/ml without metabolic activation. Positive results only at highly cytotoxic concentrations. ¹⁶⁸
Pectin-derived acidic oligosaccharides (for genotoxicity evaluation of Pectin)	F ₁ rats (from outbred strain of Wistar rats (CrI:WI(WU)))	Micronucleus test	Oral administration of diet containing pectin-derived acidic oligosaccharides (pAOS) (±7 g/kg body weight/day) for 13 weeks.	Compared to control, no increase in mean number of micronuclei in rat erythrocytes. ¹⁶⁸
Sterculia urens gum	Sprague-Dawley rats	Cytogenetic assay	Groups of rats intubated with 5,000 mg/kg, 2500 mg/kg, and 30 mg/kg, respectively. Metaphase chromosomes from rat bone marrow analyzed.	No adverse effect on rat bone marrow chromosomes. ¹⁵³
Sterculia urens gum	WI-38 human embryonic lung cells	Cytogenetic assay	up to 1,000 µg/ml	No effect on anaphase chromosomes. ¹⁵³

Table 10. Genotoxicity of Polysaccharide Gums

Ingredient/Similar Chemical	Strain/cell type	Assay	Dose	Results
Sterculia urens gum	Sprague-Dawley rats	Dominant lethal gene test	Groups of rats intubated with 5,000 mg/kg, 2500 mg/kg, and 30 mg/kg, respectively	No consistent responses suggestive of genotoxicity. ¹⁵³
Wheat bran extract (contains ~ 80% arabinoxylan) (for genotoxicity evaluation of Arabinoxylan)	Chinese hamster lung fibroblasts	Chromosome aberrations assay	up to 5,000 µg/ml, with and without metabolic activation	Not genotoxic or clastogenic. ¹⁶³

Table 11. Carcinogenicity of Polysaccharide Gums*Oral***Linear Polysaccharides and Their Salts**

Agar: 25,000 ppm or 50,000 ppm agar. Groups of 50 F344 rats and 50 B6C3F1 mice of each sex. Feeding in diet for 103 weeks. Untreated mice and rats served as controls. No clinical signs of toxicity. Increased incidence (not statistically significant) of adrenal cortical adenomas in female rats fed 50,000 ppm agar. Statistically significant increase ($p = 0.007$) in incidence of hepatocellular adenomas in male mice fed 50,000 ppm agar. Incidence of total liver tumors did not differ statistically among control, 25,000 ppm, and 50,000 ppm groups. Increased incidences of adrenal cortical adenomas and liver tumors not considered test substance-related. Agar was non-carcinogenic.¹⁸²

Algin: Up to 25% sodium alginate. Mice (75 males; 75 females). Feeding in diet for 89 weeks (dietary levels gradually increased to maximum concentration of 25%). At week 87, half of surviving male and female mice placed on control diet containing 55% pregelatinized potato starch. Algin was non-carcinogenic.¹⁶²

Carrageenan: 5% ι-carrageenan. Groups of 16 Fischer 344 rats. Feeding for up to 91 days. Proliferating cell nuclear antigen (PCNA) served as a marker of cell proliferation. Immunohistochemical staining for PCNA-positive cells in distal colon performed. Intact layer of columnar epithelial cells lining the mucosa. PCNA-positive cells not found at the luminal surface.¹⁸³

Carrageenan: 0.5%, 1.5%, and 5% ι-carrageenan. Groups of four F344 rats. Feeding in diet for 28 days. Control diet fed to additional group. Thymidine kinase enzymatic activity and PCNA served as markers of cell proliferation. No increase in PCNA-positive cells. Increased thymidine kinase levels observed only in the 5% ι-carrageenan dietary group, corresponding to a 4-fold increase in colonic cell proliferation.¹⁸³

Carrageenan: ι-carrageenan. F344 rats. Feeding in diet for 64 days, followed by 28-day recovery period. During recovery period, proliferating cells returned to level similar to those in rats fed control diet. Results suggest that the quantitative changes in cell proliferation were probably adaptive, and would not contribute to an increased risk of colon neoplasia.¹⁸³

Carrageenan: 0.1, 5, 15, and 25% carrageenan. Groups of 5 male and 5 female mice of two strains. Feeding in the diet for lifespan. Additional group fed control diet. Non-carcinogenic.¹⁸⁴

Carrageenan: 1, 5, 15, and 25% carrageenan. Groups of 5 male and 5 female mice of two strains. Feeding in diet for up to 24 months. Additional group fed control diet. Hepatic sclerosis at 25% concentration. Non-carcinogenic.¹⁸⁴

Carrageenan: 0.5, 2.5, and 5% κ-carrageenan. MRC outbred rats and randomly bred Syrian golden hamsters from the Eppley colony (30 males and 30 females per species). Average daily intake of carrageenan estimated to be 4022 mg/kg/day (rats) and 3719 mg/kg/day (hamsters) for lifetime. 100 females and 100 males per control dietary group. No increased mortality, clinical signs of toxicity, or tumor formation.¹⁸⁵

Carrageenan: Groups of female Fischer 344 rats. Co-carcinogenicity of carrageenan in presence of azoxymethane (AOM) or N-nitrosomethylurea (NMU) evaluated. Treatment groups: control diet (15 rats); 15% carrageenan in control diet (15 rats); 15% carrageenan in control diet + 10 weekly s.c. injections of 8 mg/kg bw (AOM) (30 rats); 2 mg NMU (intrarectal instillations) twice weekly for 3 weeks (30 rats); AOM s.c. alone (30 rats), and NMU i.r. alone (30 rats). Animals killed 40 weeks after the initial injection of AOM or 30 weeks after the initial injection of NMU. Carrageenan enhanced the incidence of colon tumors in AOM- and NMU-treated rats ($p < 0.01$): AOM + carrageenan (26/26, 100%) versus AOM alone (17/30, 57%); NMU + carrageenan (29/29, 100%) versus NMU alone (20/29, 69%); control diet (0/15); and 15% carrageenan in control diet (1/15, 7%).¹⁸⁶

Carrageenan: Carrageenan (0.25%, 2.5%, or 10%). Aberrant crypt focus (ACF) assay for assessment of initiation and promotion of cancer. 24 rats randomly allocated to 3 groups in initiation experiment: 9 rats given carrageenan (as a 10% jelly [24.7 g/kg body weight per day] for 8 days) in initiation experiment, 9 rats were given pure water (negative controls), and 6 rats received AOM injection (5 mg/kg i.p., positive controls). Promotion experiment: 30 rats received single azoxymethane injection (20 mg/kg i.p.) to initiate colon cancer. Seven days later, the rats were randomly allocated to the following 3 groups of 10: control group (received distilled water), group 1 (received water supplemented with 0.25% carrageenan [liquid] for 100 days), and group 2 (received water supplemented with 2.5% carrageenan [solid gel] for 100 days). In initiation experiment, no ACF found in negative controls or in rats fed carrageenan. In promotion experiment, administration of liquid 0.25% carrageenan reduced number of ACF/rat, and did not change the ACF multiplicity when compared to controls. In contrast, administration of carrageenan jelly (2.5%) for 100 days promoted growth of aberrant crypt foci ($P = 0.016$). Thus, carrageenan jelly did not initiate colon tumors; however, long-term administration of carrageenan jelly enhanced intestinal tumor growth in rats.¹⁸⁷

Carrageenan: κ-carrageenan (0.5%, 2.5%, or 10%). 54 conventional female Fischer 344 (F-344) rats (harboring a normal rat flora) and 52 germ-free female F-344 rats maintained in isolators. Initiating effect of κ-carrageenan studied by comparing number of ACF in the colon of rats given pure water or κ-carrageenan (as a 10% gel in tap water) for 8 days. Promoting effect of κ-carrageenan studied by comparing multiplicity of ACF (crypts/ACF) in rats that received pure water, liquid κ-carrageenan (0.25% in water), or κ-carrageenan gel (2.5% in water) during 100 days, beginning 7 days after a single AOM injection. κ-carrageenan (10%) did not initiate ACF. In conventional rats, the 2.5% κ-carrageenan gel promoted the growth of ACF as follows: 2.98 ± 0.29 and 3.44 ± 0.48 crypts/AF in control and treated rats, respectively ($p < 0.02$). 0.25% κ-carrageenan gel did not promote ACF.¹⁸⁸

Carrageenan: 2.5% κ-carrageenan. 8 HFA rats given κ-carrageenan and an additional 8 given water; 4 rats received AOM injection. No promotion effect: 2.81 ± 0.1 and 2.78 ± 0.38 crypts/ACF in control and treated rats, respectively ($p = 0.80$).¹⁸⁸

Carrageenan: Carrageenan (1.25%, 2.5%, or 5.0%). Groups of 18 rats or 6 rats. Groups of 18 initiated with DMH, followed by feeding with 1.25%, 2.5%, or 5% in diet for 32 weeks. Groups of 6 received saline and were then treated with 0% and 5.0% carrageenan. Detailed histopathological examination did not demonstrate any carrageenan-induced enhancement of carcinogenesis. Thus, carrageenan did not possess any promoting activity for colorectal carcinogenesis at any dietary concentration.¹⁸⁹

Carrageenan: In a monograph published by the International Agency for Research on Cancer (IARC) in 1983, IARC concluded that the available data do not provide evidence that native (undegraded) carrageenan is carcinogenic to experimental animals, and, in the absence of epidemiological data, that no evaluation of the carcinogenicity of native carrageenan in humans could be made.⁹²

Inulin: Inulin-enriched diet (10% w/w). Group of 10 to 15 Min/+ mice (has heterozygous mutation in the Apc gene, resulting in the truncated Apc protein and development of numerous intestinal adenomas.^{190,191}) fed from the age of 5 weeks to 8 or 15 weeks. Additional group fed control diet. Results indicated that dietary inulin can activate mucosal β-catenin signaling, which, in the presence of Apc mutation, induces adenoma growth.¹⁹²

Table 11. Carcinogenicity of Polysaccharide Gums

Inulin: 3 Groups of 10 Sprague-Dawley rats, consisting of control group, group treated s.c. with DMH, and group given DMH and inulin in the diet. When compared to the DMH only group, inulin in diet decreased the expression of IL-2, TNF α , and IL-10 and also decreased the numbers of COX-2- and NF κ B-positive cells in the *tunica mucosae* and *tela submucosae* of the colon. Thus, dietary intake of inulin prevented preneoplastic changes and inflammation that promote colon cancer development.¹⁹³

Inulin: Inulin (15 g) in basal diet (85 g). Groups of 20 to 22 Balb/c mice. Feeding for 7 days prior to tumor (TLT and EMT6 tumor cell lines) transplantation. Growth of both tumor cell lines significantly inhibited by supplementing the diet with inulin.¹⁹⁴

Branched - Unmodified

Arabinoxylan: Groups of 15 rats treated (s.c.) with the colon carcinogen DMH and fed either a control diet or a diet containing arabinoxylan-oligosaccharides (4.8% w/w). Two types of preneoplastic lesions (ACF and mucin-depleted foci [MDF]) detected in colon. Thirteen weeks after DMH treatment, MDF counts significantly lower in entire colon of arabinoxylan-oligosaccharides fed rats (MDF/colon were 7.5 ± 0.6 and 5.5 ± 0.6 , in control and arabinoxylan-oligosaccharides groups, respectively; means $\pm$ SE [$p = 0.05$]). Arabinoxylan-oligosaccharides fed rats had significantly fewer ACF in the distal part of the colon than control rats (ACF/distal colon were 135.5 ± 15 and 84.4 ± 11 , in control and arabinoxylan-oligosaccharides groups, respectively; means $\pm$ SE [$p = 0.05$]). Thus, dietary intake of arabinoxylan-oligosaccharides by rats reduced the occurrence of two types of preneoplastic lesions, suggesting a chemopreventive effect on colon carcinogenesis.¹⁹⁵

Arabinoxylan: Groups of 10 ICR male mice. mice were injected i.p. with mouse sarcoma S180 cells, human chronic myelogenous K562 cells, or human leukemia HL-60 cells, and dosed orally with arabinoxylan (100, 200, or 400 mg/kg body weight). All three doses conferred significant inhibitory activity against solid tumor formation in S180 tumor-bearing mice, with inhibitory ratios of 14.34%, 31.37%, and 56.73%, respectively. Arabinoxylan did not have any effect on growth of K562 or HL-60 cells *in vitro*.¹⁹⁶

Glucomannan: 10% Glucomannan. Groups of 30 C3H/He male mice fed either a powdered commercial diet (control group) or the same diet containing 10% glucomannan. At age 1 year, slight decrease in the number of animals with liver tumors in glucomannan group (control: 63% of 24 mice; glucomannan: 48% of 23 mice) and a statistically significant decrease ($p < 0.05$) in the mean number of tumor nodules per mouse in the glucomannan group (control: 1.1; konjac mannan: 0.5). Thus, spontaneous liver tumors in C3H/He mice were inhibited by maintaining the mice on a diet containing 10% glucomannan.¹⁹⁷

Glucomannan: 5% Glucomannan. Fisher 344 rats (20/group) fed either a commercial diet or similar diet containing 5% glucomannan for 13 weeks. Animals also injected i.p. with DMH weekly. Incidence of DMH-induced colon tumors significantly lower in glucomannan-fed group (39%) when compared to control group (75%). Number of colon adenocarcinomas per rat also significantly lower in glucomannan-fed rats (0.22) than in control rats (0.75). No significant effect on the incidence of tumors of the small intestine, all of which were adenocarcinoma (control: 45%; konjac mannan: 33%).¹⁹⁸

Pectin: 2.5% Pectin. Male Wistar rats (groups of 4). Feeding in diet for 14 days. Statistically significant increase in the villus height and crypt depth, indicating that feeding with pectin caused mucosal hyperplasia in small intestine.¹⁹⁹

Starch Acetate: 55% Starch Acetate. Mice (75 males, 75 females) fed starch acetate in diet for 89 weeks. Dietary levels of the test substance gradually increased until diet contained (by weight) 55% starch acetate. At week 87, half of surviving male and female mice placed on control diet (containing 55% pregelatinized potato starch). No evidence of carcinogenicity.¹⁶²

Cyclic

Cyclodextrin: 2.5% or 5% β -cyclodextrin. 2 groups of Fischer 344 (F344) rats (50 males and 50 females/group) fed 2.5% and 5% β -cyclodextrin, respectively, for 104 weeks. Control diet fed to additional group. All neoplastic lesions observed were histologically similar to those known to occur spontaneously in this strain of rat; no statistically significant increase in the incidence of any tumor found for either sex in treated groups. It was concluded that the high dose, which was approximately 340-400 times higher than the current daily human intake from ingestion as a food additive and from pharmaceutical use, did not have carcinogenic potential in F344 rats.¹¹¹

Cyclodextrin: β -cyclodextrin. 5 groups of 50 Fischer 344 rats and 52 CD-1 outbred mice of each sex. 4 groups per strain received β -cyclodextrin in the diet at doses of 25, 75, 225, and 675 mg/kg per day, respectively for 93 weeks (males) and between weeks 129 and 130 (females). Fifth group received control diet. No treatment-related carcinogenic effects.²⁰⁰

Degraded Polysaccharide Gum

Degraded Carrageenan: Degraded carrageenan (from *Eucheuma spinosum*; degraded by acid hydrolysis). 4 groups of 30 males and 30 female rats fed a diet containing 0 (control), 1%, 5%, or 10% degraded carrageenan. Colorectal squamous metaplasia in rats fed degraded carrageenan at concentrations of 10% (59 of 60 rats) and 5% (53 of 60 rats) in the diet. Additionally, colorectal tumors (12 squamous-cell carcinomas, 8 adenocarcinomas and 3 adenomas) found in 19 of 60 rats fed 10% degraded carrageenan in the diet, and these tumors (3 squamous-cell carcinomas, 1 adenocarcinoma and 8 adenomas) also found in 12 of 60 rats fed 5% degraded carrageenan. Neither squamous metaplasia nor colorectal tumors observed in the low-dose group or in controls.⁹²

Degraded Carrageenan: Degraded carrageenan (5% in drinking water) administered to 20 male and 20 female rats for 15 months. Colorectal squamous metaplasia observed in all rats after 15 months. Colorectal tumors observed in 11 of 40 treated rats (4 squamous-cell carcinomas, 4 adenocarcinomas, 3 adenomas and 1 myosarcoma); these tumors not observed in control rats (15 males, 15 females).²⁰¹

Degraded Carrageenan: Degraded carrageenan (1 or 5 g/kg body weight) administered by intragastric intubation (frequency of administration not specified) to groups of 15 male and 15 female rats for 15 months. Control rats (15 males, 15 females) dosed intragastrically with distilled water. Squamous colorectal metaplasia observed in all 29 rats in high-dose group and in 11 of 30 rats in low-dose group. Colorectal tumors were observed only in the high-dose group (8 of 29 rats; 5 adenocarcinomas and 4 adenomas).²⁰²

Degraded Carrageenan 10% degraded carrageenan (in diet that also contained 30% sulfate) fed to Fischer 344 rats. Three groups fed this diet for 2 months (39 rats, group 1), 6 months (42 rats, group 2), and 9 months (42 rats, group 3). Control group (46 rats) received the same diet without carrageenan, and the same was true for all other groups after cessation of feeding. 100% incidence of colorectal squamous metaplasia observed in all treatment groups. Tumors also observed in 5 of 39 rats in group 1 (3 squamous-cell carcinomas, 1 adenoma, 1 anaplastic carcinoma), 8 of 42 rats in group 2 (6 squamous-cell carcinomas, 1 adenocarcinoma, 1 adenoma) and in 17 of 42 rats in group 3 (14 squamous-cell carcinomas, 4 adenocarcinomas). Colorectal changes not observed in control rats.^{92,203}

Table12. Skin Irritation/Sensitization Potential of Polysaccharide Gums*Skin Irritation and Sensitization - Non-Human***Linear Polysaccharides and Their Salts**

Algin: 2% algin. Rabbits (number not stated). 3 primary skin irritation experiments. Occlusive patches applied to the skin. Mean skin irritation score of < 0.5 = non-irritating; 0.5 to 2.0 = slightly irritating. Primary irritation index (PII) values calculated. PII of < 0.5 deemed satisfactory, but PII no greater than 1 is also acceptable. PII values of 0, 0, and 0.08 were reported in the 3 experiments, respectively.⁶¹

Algin: 2% algin. Rabbits (3 per experiment). Test substance (2 ml) applied to flanks 5 days per week for 6 weeks. Mean maximum irritation index (MMII) values calculated. Macroscopic and histological examinations of test sites performed. MMII values of 0.67, 0, and 0.67 were reported in 3 experiments, respectively. Daily application of test substance did not induce a severe reaction at either macroscopic or histological examination.⁶¹

Carrageenan: Food grade iota-carrageenan (one subtype of carrageenan with a specific number and position of sulfate groups on the repeating galactose units). Guinea pigs (number not stated). Study details not included. No skin sensitization.⁶²

Branched - Unmodified

Glucomannan (in konjac flour [mechanically ground]). Guinea pigs (number not stated). Application to skin according to the Buehler closed patch method. No sensitization.¹⁵¹

Branched - Modified

Corn Starch Modified: Corn starch modified in distilled water (30% solids). 10 Zealand White rabbits (5 males and 5 females). Application to skin (2,000 mg/kg); dose per cm² not stated. Dermal reactions either absent or classified as barely perceptible at 24-h and 48-h readings, and absent at the 74-h reading. Mild skin irritant (primary irritation index = 0.25).⁶⁶

Corn Starch Modified: Corn Starch Modified (up to 30%). 20 guinea pigs (strain not stated; 10 males, 10 females). Maximization test (OECD protocol 406.) During induction, 10% solution injected and 30% solution applied topically. Concentration per cm² was not stated. During challenge, application of 20% solution for 24 h. Reactions scored at 48 h and 72 h post-application. Control group (5 males, 5 females) tested with distilled water during induction and challenged with test substance. Reactions ranging from no erythema to moderate erythema observed after induction with the control or test substance. Erythema observed after challenge with test substance. However, rechallenge with same test substance concentration did not cause erythema. Not a sensitizer.⁶⁶

Corn Starch Modified: 50% corn starch modified paste. 25 female Hartley guinea pigs. RIPT according to Buehler method (OECD protocol 4067). 10 guinea pigs treated with distilled water (control). Positive control (isoeugenol) tested in study performed within 6 months of current study. During induction, test material applied topically to shoulder area (~ 0.4 g on occlusive patch; area of application site not stated). Topical challenge with 50% corn starch modified paste for 6 h. Challenge reactions scored at 24 h and 48 h post-application. No erythema or edema during induction or challenge. Non-sensitizer. Positive control induced sensitization.⁶³

Carboxymethyl Inulin: Carboxymethyl inulin (1% to 100%). Groups of 2 adult Dunkin–Hartley albino guinea pigs. Test substance injected into clipped scapular region; reactions scored at 24 h and 48 h. Also, series of test article concentrations (0.5 ml) applied topically for 24 h to clipped external flank using Metalline patches secured with tape and an elastic bandage. Test material was removed after 24 h and signs of irritation recorded at 24 h and 48 h after treatment. Undiluted carboxymethyl inulin produced necrosis after intradermal injection, observed both after 24 h and 48 h; 20% to 50% did not cause necrosis, but grade 2 erythema was observed at either 24 h or 48 h. Signs of irritation were not observed at 24 h or 48 h at concentrations up to 100% in the patch tests.¹⁵⁶

Carboxymethyl Inulin: 31.1% aqueous carboxymethyl inulin. 10 adult Dunkin–Hartley albino guinea pigs. Maximization test. Five female guinea pigs served as vehicle controls. No evidence of sensitization.¹⁵⁶

Potato Starch Modified: 10 rats received single dose of potato starch modified (dose = 2 g/kg) dermally. Very slight to well-defined erythema and edema observed in all animals after 24 h. At 48 h, very slight erythema and very slight edema in 5 and 3 rats, respectively. All reactions had cleared by 72 h.¹⁵⁵

Potato Starch Modified: Rats (10 males, 10 females). Dose of 2 g/kg body weight/day applied to the skin, under occlusive dressing, for 28 days. Neither erythema nor edema observed. However, small scabs observed on 5 males and 6 females, attributed to adhesion of test material to skin.¹⁵⁵

Potato Starch Modified: Potato starch modified (18.5% aqueous suspension). 20 guinea pigs. Buehler test (OECD 406 test guideline). Faint erythema (non-confluent) observed in 6 of 20 animals after second or third induction application. No evidence of sensitization.¹⁵⁵

Potato Starch Modified: Potato Starch Modified (10% solids aqueous solution). 10 male and 10 female New Zealand albino rabbits (test animals). Using non-occlusive patch, test substance (2 g/kg body weight) applied to the skin. The area of application and dose per cm² not stated. 20 control animals tested with distilled water under non-occlusive patch. Neither erythema nor edema observed in treated or control animals. No adverse morphologic effects on the skin.⁷⁰

Potato Starch Modified: Potato starch modified (18.5% solids). 20 guinea pigs (10 males, 10 females). RIPT according to Buehler method (OECD 406 protocol). Concentration per cm² not stated. 10 control animals (5 males, 5 females) treated with distilled water. During induction, very faint erythema in 6 of 20 animals; reactions not observed in controls. Very faint erythema observed in 2 of 20 treated animals and in 2 of 10 controls during challenge phase. Non-sensitizer.⁷⁰

Dextrin Myristate: 6 New Zealand white rabbits. Skin irritation study (test protocol not stated). Non-irritant.⁶⁷

Dextrin Myristate: Guinea pigs (number and strain not stated). Magnusson-Kligman maximization test. No evidence of skin sensitization.⁶⁷

Dextrin Palmitate: 3 New Zealand white rabbits. Skin irritation study (test protocol not stated). Non-irritant.^{68,69}

Table12. Skin Irritation/Sensitization Potential of Polysaccharide Gums**Branched - Modified**

Dextrin Palmitate: Guinea pigs (number and strain not stated). Magnusson-Kligman maximization test (test concentrations not stated). No evidence of skin sensitization.^{68,69}

Sodium Hydrolyzed Potato Starch Dodecenylsuccinate: Test Material: Material (corn starch modified) described as structurally similar to sodium hydrolyzed starch dodecenylsuccinate and as the calcium salt of the ester formed from the reaction of 3-(dodecenyl)dihydro-2,5-furandione and corn starch, in which the degree of substitution per glucose unit is less than 0.1. 6 New Zealand White rabbits. OECD 404 test protocol. 50% slurry of test material (1 ml) applied topically (on occlusive patch, area of application site not stated) for 24 h to intact and abraded skin sites on the back of each animal. Reactions scored for up to 72 h after patch application. Erythema observed at intact and abraded sites on one animal, and reactions had cleared by 48 h. Mildly irritating to the skin (primary irritation index = 0.09).^{63,204}

Stearoyl Inulin: 6 Japanese white rabbits. Skin irritation potential evaluated (concentrations and test protocol not stated). Non-irritant.^{71,72}

Stearoyl Inulin: Guinea pigs (number and strain not stated). Skin sensitization potential evaluated (concentrations not stated) according to adjuvant and patch method. Skin irritation classified as weak. Very low skin sensitization potential.^{71,72}

*Skin Irritation and Sensitization - Human***Linear Polysaccharides and Their Salts**

Algin: 20% aqueous sodium alginate. 12 male subjects with no history of allergy. Patch-testing (Finn chambers) with 20% aqueous sodium alginate according to International Contact Dermatitis Research Group (ICDRG) recommendations. Area (cm²) of application and dose per cm² not stated. Reactions scored at 2 and 3 days post-application. ± reaction observed in one subject on days 2 and 3. Results negative for skin irritation and allergic contact dermatitis.²⁰⁵

Linear - Modified

Hydrolyzed Furcellaran: Mixture containing 1.35% furcellaran powder and 1% phenoxyethanol. 10 adults. Mixture applied (under occlusive patch) for 48 h to back. Area (cm²) of application and dose per cm² not stated. Non-irritant.⁷³

Hydrolyzed Furcellaran: Mixture containing 1.35% furcellaran powder, 0.1% potassium sorbate, and 0.05% citric acid. 10 adults. Mixture applied (under occlusive patch) for 48 h to back. Area (cm²) of application and dose per cm² not stated. Non-irritant and non-sensitizer.⁷³

Hydrolyzed Furcellaran: Mixture containing 0.6% hydrolyzed furcellaran, 0.05% concentrate of sea water, 1% phenoxyethanol, and 98.35% water. 100 subjects. Mixture applied 9 times to each subject. Area (cm²) of application and dose per cm² not stated. Non-irritant and non-sensitizer.⁷³

Maltodextrin: Eye gel containing 2.45% maltodextrin. 103 subjects. HRIPT. Patch type, area (cm²) of application, and dose per cm² not stated. Challenge patches applied to original and alternate sites, and challenge reactions scored at approximately 48 h and 96 h post-application. Five instances of erythema (grade 1) during induction. At 48-h challenge reading, a grade of 1 reported for alternate challenge site of one subject. Gel did not induce allergic contact dermatitis.²⁰⁶

Branched - Modified

Corn Starch Modified: 7.5% solution in distilled water. 26 female subjects. 21-day cumulative irritation study. Test material (0.2 ml per 24-h patch) applied topically. Area (cm²) of application and dose per cm² not stated. Reactions ranged from no erythema to minimal erythema. Non-irritant. Distilled water (vehicle control) did not cause erythema. Sodium lauryl sulfate (positive control) induced marked erythema and papules.⁶⁶

Corn Starch Modified: 7.5% solution in distilled water. 113 subjects (86 females, 27 males). HRIPT. Patch type, area (cm²) of application, and dose per cm² not stated. Challenge reactions scored at 48 h and 96 h post-application. Test substance and distilled water caused slight erythema in 3 subjects. Test substance and distilled water classified as non-sensitizers.⁶⁶

Dextrin: Rinse-off facial product containing 42.6919 % dextrin (1% aqueous; effective concentration ≈ 0.4%). 54 subjects (46 females, 8 males). HRIPT. During induction, product (0.1-0.15 g on occlusive patch) applied for 24 h to the back. Dose/concentration per cm² not stated. Challenge patch applied to new test site and reactions scored at 24 h and 72 h post-application. Transient, barely perceptible erythema, in 1 subject, during induction. No reactions observed during challenge phase. No clinically significant skin irritation or evidence of allergic contact dermatitis.²⁰⁷

Dextrin Myristate: Leave-on facial product containing 0.3% dextrin myristate. 51 subjects (40 females, 11 males). HRIPT. During induction, product (0.1-0.15 g on occlusive patch) applied for 24 h to the back. Dose/concentration per cm² not stated. Challenge patch applied to new test site and reactions scored at 24 h and 72 h post-application. Skin reactivity was not observed during the induction or challenge phase. Product did not cause skin irritation or allergic contact dermatitis.²⁰⁸

Hydroxypropyltrimonium Hydrolyzed Corn Starch: 15% hydroxypropyltrimonium hydrolyzed corn starch. 47 male and female subjects. HRIPT. During induction, semi-occlusive patch (1" x 1") containing approximately 0.2 ml of test material applied for 24 h to upper back. 24-h challenge patch applied to new test site, adjacent to induction patch site. No reactions during study. No skin irritation or allergic contact sensitization potential.²⁰⁹

Calcium Starch Isododecenylsuccinate: Test material (powder) and a 50% w/v slurry of test material in baby oil tested. 23 subjects. Powder applied topically (0.2 g, moistened with distilled water; area of application site not stated) under occlusive conditions for 21 days. 50% w/v slurry applied according to same procedure. Powder caused dermal effects that ranged from no irritation to erythema and papules. Cumulative irritation score = 177). Superficial layer effects ranged from none to glazing with peeling and cracking. 50% w/v slurry caused milder reactions (cumulative irritation score = 50.6). Both test materials classified as probable mild irritants under normal use conditions.^{63,64,210}

Branched - Modified

Sodium Hydrolyzed Potato Starch Dodecenylsuccinate: Cleanser containing 10 wt% sodium hydrolyzed potato starch dodecenylsuccinate. 227 subjects (18 to 69 years old; 165 females, 62 males). HRIPT. During induction, occlusive patch containing ~ 0.2 g of the test material was applied to the back (area of application site not stated) for 24 h. week non-treatment period. Occlusive challenge patch containing the test material (~ 0.2 g) applied for 24 h to new site on back. Reactions were scored for up to 96 h post-application. Four subjects had low-level (±) reactions during induction, and 2 subjects had ± reactions during challenge phase. Non-sensitizer.²¹¹

Table12. Skin Irritation/Sensitization Potential of Polysaccharide Gums

Unknown Structural Configuration

Algae Exopolysaccharides: 1% solution of algae exopolysaccharides. 50 subjects. HRIPT. During induction, occlusive patch containing test substance (0.2 ml or 0.2 g) applied for 24 h to infrascapular region of back. Dose per cm² not stated. Challenge dose (equivalent to induction application) of test substance applied once to new test site. Reactions scored at 24 h to 48 h post-application. No evidence of adverse reactions. Not a primary skin irritant or sensitizer.²¹²

In Vitro

Branched - Modified

Hydroxypropyltrimonium Hydrolyzed Corn Starch: MatTek Corporation EpiDermTM skin model *in vitro* toxicity testing system. Skin model consists of normal, human-derived epidermal keratinocytes (NHEK) that have been cultured to form a multilayered, highly differentiated model of the human epidermis. Test procedure utilizes a water-soluble, yellow tetrazolium salt MTT. In the mitochondria of viable cells, MTT is reduced by succinate dehydrogenase to an insoluble formazan derivative (purple color). Substances that damage this enzyme inhibit reduction of the tetrazolium salt. Undiluted test substance (100 µl) added to millicells containing EpiDermTM samples; time at which % viability would be 50% (ET₅₀) estimated. Mild irritant (ET₅₀ = 18.1h).²¹³

References

1. Nikitakis, J. and Breslawec H. P. International Cosmetic Ingredient Dictionary and Handbook. 14^{ed}. Washington, DC: Personal Care Products Council, 2014.
2. Johnson, W. Jr. Bergfeld W. F. Belsito D. V. Marks J. G. Jr. Shank R. C. Slaga T. J. Snyder P. W. Klaassen C. D. Liebler D. C. Hill R. A. and Andersen F. A. 2012. Final Report on the safety assessment of galactomannans. Unpublished report. Washington: Cosmetic Ingredient Review.
3. Fiume, M. M. Heldreth B. A. Bergfeld W. F. Belsito D. V. Hill R. A. Klaassen C. D. Liebler D. C. Marks J. G. Jr. Shank R. C. Slaga T. J. Snyder P. W. and Andersen F. A. 2012. Final safety assessment of microbial polysaccharide gums as used in cosmetics. Unpublished report. Washington:Cosmetic Ingredient Review.
4. Elder, RL. Final report on the safety assessment of tragacanth gum. *Journal of the American College of Toxicology*. 1987;6(1):1-22.
5. Andersen, FA. Annual review of cosmetic ingredient safety assessments - 2004/2005. Astragalus gummifer gum. *International Journal of Toxicology*. 2006;25(2):1-89.
6. Bergfeld, WFB DVSPWKCDMJGJrSRCSTJaAFA. Final report on the safety assessment of aloe andongensis extract, aloe andongensis leaf juice, aloe arborescens leaf extract, aloe arborescens leaf juice, aloe arborescens leaf protoplasts, aloe barbadensis flower extract, aloe barbadensis leaf, aloe barbadensis leaf extract, aloe barbadensis leaf juice, aloe barbadensis leaf polysaccharides, aloe barbadensis leaf water, aloe ferox leaf extract, aloe ferox leaf juice, and aloe ferox leaf juice extract. *International Journal of Toxicology*. 2007;26(2):1-50.
7. Bergfeld, WFB DVSPWKCDMJGJrSRCSTJaAFA. Amended final report on the safety assessment of oryza sativa (rice) bran oil, oryza sativa (rice) germ oil, rice bran acid, oryza sativa (rice) bran wax, hydrogenated rice bran wax, oryza sativa (rice) bran extract, oryza sativa (rice) extract, oryza sativa (rice) germ powder, oryza sativa (rice) starch, oryza sativa (rice) bran, hydrolyzed rice bran extract, hydrolyzed rice bran protein, hydrolyzed rice extract, and hydrolyzed rice protein. *International Journal of Toxicology*. 2006;25(2):91-120.
8. Andersen, FABWFB DVKCDMJGJrSRCSTJaSPW. Final report of the safety assessment of cosmetic ingredients derived from zea mays (corn). *International Journal of Toxicology*. 2011;30(1):17S-39S.
9. Bergfeld, WFB DVSPWKCDMJGJrSRCSTJaAFA. Final report on the safety assessment of acacia catechu gum, acacia concinna fruit extract, acacia dealbata leaf extract, acacia dealbata leaf wax, acacia decurrens extract, acacia farnesiana extract, acacia farnesiana flower wax, acacia farnesiana gum, acacia senegal extract, acacia senegal gum, and acacia senegal gum extract. *International Journal of Toxicology*. 2005;24(3):75-118.
10. Johnson, WJrBWFBDVMJGJrSRCSTJSPWKCDaAFA. Final report of the amended safety assessment of glyceryl laurate, glyceryl laurate SE, glyceryl laurate/oleate, glyceryl adipate, glyceryl alginate, glyceryl arachidate, glyceryl arachidonate, glyceryl behenate, glyceryl caprate, glyceryl caprylate, glyceryl caprylate/caprate, glyceryl citrate/lactate/linoleate/oleate, glyceryl cocoate, glyceryl collagenate, glyceryl erucate,

- glyceryl hydrogenated rosinat, glyceryl hydrogenated soyate, glyceryl hydroxystearate, glyceryl isopalmitate, glyceryl isostearate, glyceryl isostearate/myristate, glyceryl isostearates, glyceryl lanolate, glyceryl linoleate, glyceryl linolenate, glyceryl montanate, glyceryl myristate, glyceryl isotridecanoate/stearate/adipate, glyceryl oleate SE, glyceryl oleate/elaidate, glyceryl palmitate, glyceryl palmitate/stearate, glyceryl palmitoleate, glyceryl pentadecanoate, glyceryl polyacrylate, glyceryl rosinat, glyceryl sesquioleate, glyceryl sorbitol/oleate/hydroxystearate, glyceryl stearate/acetate, glyceryl stearate/maleate, glyceryl tallowate, glyceryl thiopropionate, and glyceryl undecylenate. *International Journal of Toxicology*. 2006;25(2):55-94.
11. Becker, LCBWFBVDKCDMJGJrSRCSTJSPWaAFA. Final report of the safety assessment of hyaluronic acid, potassium hyaluronate, and sodium hyaluronate. *International Journal of Toxicology*. 2009;28(1):5-67.
 12. Elder, RL. Final report of the safety assessment for wheat flour and wheat starch. *Journal of Environmental Pathology and Toxicology*. 1980;4(4):19-32.
 13. Andersen, FA. Annual review of cosmetic ingredeint safety assessments - 2001/2002. *International Journal of Toxicology*. 2003;22(1):1-35.
 14. Anonymous. Manufacturing process for hydrolyzed furcellaran. Unpublished data submitted by the Personal Care Products Council on 9-22-2014. 2014. pp.1
 15. Anonymous. Chemical structure diagram and descriptive information for production of: starch, 2-hydroxypropyl, oxidized succinic acid ester (sodium hydroxypropyl oxidized starch succinate). Unpublished data submitted by the Personal Care Products Council on 11-13-2014. 2014. pp.1
 16. Personal Care Products Council. Concentration of use by FDA product category: Plant polysaccharides. Unpublished data submitted by the Personal Care Products Council on 3-21-2013. 2013. pp.1-22.
 17. Food and Drug Administration (FDA). Information supplied to FDA by industry as part of the VCRP FDA database. 2015. Washington, D.C.: FDA.
 18. Personal Care Products Council. Concentration of use by FDA product category: Glucomannan. Unpublished data submitted by the Personal Care Products Council on 1-5-2015. 2015. pp.1
 19. Personal Care Products Council. Concentration of use by FDA product category: sodium hydrolyzed potato starch dodecenylsuccinate. Concentration of use survey: No reported uses of sodium hydrolyzed potato starch dodecenylsuccinate. Unpublished data submitted by the Personal Care Products Council on 4-8-2015. 2015. pp.1
 20. Rothe H, Fautz R, Gerber E, Neumann L, Rettinger K, Schuh W, and Gronewold C. Special aspects of cosmetic spray safety evaluations: Principles on inhalation risk assessment. *Toxicol Lett*. 2011;205(2):97-104. PM:21669261.
 21. Bremmer HJ, Prud'homme de Lodder LCH, and van Engelen JGM. Cosmetics Fact Sheet: To assess the risks for the consumer; Updated version for ConsExpo 4. 20200. <http://www.rivm.nl/bibliotheek/rapporten/320104001.pdf>. Date Accessed 8-24-2011. Report No. RIVM 320104001/2006. pp. 1-77.

22. Rothe H. Special aspects of cosmetic spray evaluation. 2011.
23. Johnsen MA. The Influence of Particle Size. *Spray Technology and Marketing*. 2004;14(11):24-27. <http://www.spraytechnology.com/index.mv?screen=backissues>.
24. Food and Drug Administration (FDA). GRAS Substances (SCOGS) Database [Food]. <http://www.fda.gov/food/ingredientpackaginglabeling/gras/scogs/default.htm>. Last Updated 2014. Date Accessed 9-30-2014.
25. Food and Drug Administration (FDA). Everything added to food in the United States (EAFUS). <http://www.accessdata.fda.gov/scripts/fcn/fcnNavigation.cfm?rpt=eafusListing>. Last Updated 2014. Date Accessed 9-30-2014.
26. Zhao, LYGLFKXWBSQXHHDaZH. Sodium alginate as viscosity modifier may induce aggregation of red blood cells. *Artificial Cells, Blood Substitutes, and Biotechnology*. 2010;38:267-276.
27. Sweeney, IRMMaCG. A critical review of modern and emerging absorbent dressings used to treat exuding wounds. *Int.Wound J.* 2012;9:601-612.
28. Amaguana, MRHTSaAWH. Methods for the recovery of *Salmonella* spp. from carboxymethylcellulose gum, gum ghatti, and gelatin. *Journal of AOAC International*. 1998;81(4):721-726.
29. World Health Organization. Joint FAO/WHO Expert Committee on Food Additives. Seventy-ninth meeting. Geneva, 17-26 June 2014. Summary and Conclusions. Carrageenan for use in infant formula and formula for special medical purposes intended for infants. <http://www.fao.org>. Last Updated 2014.
30. Kaur, NaGA. Applications of inulin and oligofructose in health and nutrition. *J.Biosci.* 2002;27:703-714.
31. Food and Drug Administration (FDA). Everything added to food in the United States (EAFUS). Listing of specific substances affirmed as GRAS. Karaya gum (sterculia gum). 21CFR:184.1349. www.fda.gov. Last Updated 2014.
32. World Health Organization (WHO). Seventeenth Report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series No. 539. FAO Nutrition Meeting Series No. 53. Geneva: World Health Organization, 1974.
33. World Health Organization (WHO). Evaluation of certain food additives and contaminants, JECFA 27. Technical Report Series No. 696. Geneva: World Health Organization, 1983.
34. Loftsson, THDaMM. Evaluation of cyclodextrin solubilization of drugs. *Internatioinal Journal of Pharmaceuticals*. 2005;302(1-2):18-28.
35. Cohen, SMaIN. A critical review of the toxicological effects of carrageenan and processed Eucheuma seaweed on the gastrointestinal tract. *Critical Reviews in Toxicology*. 2002;32(5):413-444.
36. Brown, PMPMAaADMW. A study of the fate of gum karaya in the rat. *Toxicology Letters*. 1982;13:247-251.

37. Gerloczy, AFAKPPLaSJ. Absorption, distribution, excretion, and metabolism of orally administered ^{14}C -beta-cyclodextrin in rat. *Arzneim.Forsch.* 1985;35(7):1042-1047.
38. Behne, MTHBDHLVAFaFH. The pharmacokinetics of acetyl starch as a plasma volume expander in patients undergoing elective surgery. *Anesth.Analg.* 1998;86(856):860
39. Anderson, AWJBWJEMAMFJaADMW. The absence of rhamnose in human urine following the ingestion of gum karaya (*Sterculia*). *Food Additives and Contaminants.* 1985;2(1):33-36.
40. Tachibe, MOHNTaEK. Digestibility, fermentability, and energy value of highly cross-linked phosphate tapioca starch in men. *Journal of Food Science.* 2011;76(6):H152-H155.
41. Tanaka, MIYKYTKMHAHaTS. Effect of 2-hydroxypropyl- β -cyclodextrin on percutaneous absorption of methyl paraben. *J.Pharm.Pharmacol.* 1995;47:897-900.
42. World Health Organization (WHO). Toxicological evaluation of certain food additives and contaminants. β -Cyclodextrin. The forty-first meeting of the Joint FAO/WHO Expert Committee on Food Additives. WHO Food Additive Series 32. 1993. pp.173-193. Geneva: World Health Organization.
43. Olivier, PVFaHAR. Subchronic toxicity of orally administered β -cyclodextrin in rats. *J.Am.Coll.Toxicol.* 1991;10:407-419.
44. Bellringer, MLSTGRRGCaOP. β -Cyclodextrin: 52-week toxicity studies in the rat and dog. *Fd.Chem.Toxic.* 1995;33(5):367-376.
45. Fromming, K. H. and Szejtli J. Pharmacokinetics and toxicology of cyclodextrins. Chapter: 3. Szejtli, J. and Szenté L. In: *Proc. of the 8th Int. Symposium on Cyclodextrins.* 1996:33-45.
46. Perrin, JHFFPHDAMRAaTG. β -cyclodextrin as an aid to peritoneal dialysis. Renal toxicity of β -cyclodextrin in the rat. *Res.Commun.Chem.Pathol.Pharmacol.* 1978;19:373-376.
47. Frank, DWGJEaWRN. Cyclodextrin nephrosis in the rat. *Am.J.Pathol.* 1976;83:367-382.
48. Gong, YHGZYPYLxXYaWY. Antifungal activity and cytotoxicity of zinc, calcium, or copper alginate fibers. *Biol.Trace.Elem.Res.* 2012;148:415-419.
49. Majimal, ROKNRaKT. Polysaccharide (ANK-102) from *Polianthes tuberosa* cells deteriorates the resistance of mice to *Listeria monocytogenes* infection. *Immunopharmacology and Immunotoxicology.* 1995;17(1):59-68.
50. Fowler, EFaTAW. Serum IgG, IgM, and slow α -globulin levels in carrageenan-treated rats. *Br.J.Exp.Pathol.* 1979;60:353-357.
51. Chatterjee, MLaDMS. Gum karaya (*Sterculia urens* Roxb. Family: Sterculiaceae) allergenic? *Bull.Cal.S.T.M.* 1966;14(1):10-11.
52. Strobel, SFAaADMW. Immunogenicity, immunological cross reactivity and non-specific irritant properties of the exudate gums, arabic, karaya, and tragacanth. *Food Additives & Contaminants.* 1986;3(1):47-56.

53. Anderson, DMWBWGEMAAaSDM. Dietary effects of propylene glycol alginate in humans. *Food Additives and Contaminants*. 1991;8(3):225-236.
54. Sennerby, LRTABaAT. Acute tissue reactions to potassium alginate with and without colour/flavour additives. *Biomaterials*. 1987;8:49-52.
55. MB Research Laboratories. 3T3 neutral red uptake phototoxicity assay. Structure PS-111. Unpublished data submitted by AkzoNobel on 4-14-2014. 2013. pp.1-16.
56. Ausili, EPVTCMDMDFBaRC. Treatment of pressure sores in spina bifida patients with calcium alginate and foam dressings. *European Review for Medical and Pharmacological Sciences*. 2013;17:1642-1647.
57. Bhalla, RKMJJTMaRNJ. Foreign body reaction to calcium alginate fibre mimicking recurrent tumour of the submandibular gland. *British Journal of Oral and Maxillofacial Surgery*. 2002;40:172-174.
58. Moulonguet, IdGCaPF. Granulomatous reaction after injection of a new resorbable filler Novabel. *Am.J.Dermatopathol*. 2011;33(7):710-711.
59. Schuller-Petrovic, SPMDSSsS-LBaNN. Early granulomatous foreign body reactions to a novel alginate dermal filler: the system's failure? *JEADV*. 2011;27:121-123.
60. Tarlo, SMDJaLC. Anaphylaxis to carrageenan: A pseudo-latex allergy. *J.Allergy Clin.Immunol*. 1995;95:933-936.
61. Guillot, JPGJYMMCGJFaSG. Safety evaluation of gums and thickeners used in cosmetic formulations. *International Journal of Cosmetic Science*. 1982;4:53-66.
62. World Health Organization. Safety evaluation of certain food additives. Carrageenan. Prepared by the fifty-first meeting of the Joint FAO/WHO Expert Committee of Food Additives (JECFA). WHO Food Additives Series 42. <http://www.inchem.org/documents/jecfa/jecmono/v042je08.htm>. Last Updated 1999. Date Accessed 9-4-2014.
63. AkzoNobel. Toxicology summary. Sodium hydrolyzed starch dodecenylsuccinate. Unpublished data submitted by AkzoNobel on 4-11-2014. 2014. pp.1-2.
64. AkzoNobel. Toxicology summary. Corn-starch modified. Unpublished data submitted by AkzoNobel on 4-11-2014. 2014. pp.1-2.
65. MB Research Laboratories, Inc. Eye irritation in albino rabbits. Test article: 73-8050. Unpublished data submitted by AkzoNobel on 4-14-2014. 1996. pp.1-10.
66. Akzo Nobel. Toxicology summary on Amaze® (corn starch modified). Unpublished data submitted by the Personal Care Products Council on 11-12-2014. 2014. pp.1-3.
67. Chiba Flour Milling Co., Ltd. Material safety data sheet on Rheopearl MKL2 (dextrin myristate). Unpublished data submitted by the Personal Care Products Council on 10-10-2014. 2010. pp.1-7.

68. Chiba Flour Milling Co., Inc. Material safety data sheet on Rheopearl KL2 (dextrin palmitate). Unpublished data submitted by the Personal Care Products Council on 10-10-2014. 2011. pp.1-7.
69. Chiba Flour Milling Co., Ltd. Material safety data sheet on Rheopearl TL2 (dextrin palmitate). Unpublished data submitted by the Personal Care Products Council on 10-10-2014. 2011. pp.1-7.
70. Personal Care Products Council. Potato starch modified. Method of manufacture and safety study summaries. Unpublished data submitted by the Personal Care Products Council on 10-23-2014. 2014. pp.1-2.
71. Chiba Flour Milling Co., Ltd. Material safety data sheet on Rheopearl ISK2 (stearoyl inulin). Unpublished data submitted by the Personal Care Products Council on 10-10-2014. 2010. pp.1-7.
72. Chiba Flour Milling Co., Ltd. Material safety data sheet on Rheopearl ISL2 (stearoyl inulin). Unpublished data submitted by the Personal Care Products Council on 10-10-2014. 2010. pp.1-7.
73. Phycher Bio developpement. Toxicological expertise of hydrolyzed furcellaran. Unpublished data submitted by the Personal Care Products Council on 9-22-2014. 2009. pp.1-6.
74. Thomas J.Stephens & Associates, Inc. Bovine corneal opacity and permeability assay on an eye gel containing 2.45% maltodextrin. Unpublished data submitted by the Personal Care Products Council on 6-26-2014. 1996. pp.1-7.
75. Thomas J.Stephens & Associates, Inc. Matek Corporation epi-ocular® skin model on an eye gel containing 2.45% maltodextrin. Unpublished data submitted by the Personal Care Products Council on 6-26-2014. 1996. pp.1-7.
76. Consumer Product Testing Co. The hen's egg test - utilizing the chorioallantoic membrane (HET-CAM): Hydroxypropyltrimonium hydrolyzed corn starch. Unpublished data submitted by the Personal Care Products Council on 11-21-2014. 2005. pp.1-7.
77. Konjac Foods. Benefits and nutritional information about Konjac. <http://www.konjacfoods.com/health/index.html>. Last Updated 2002. Date Accessed 10-24-2014.
78. Werley, MSB-FH and Mount, EAaKLA. Respiratory sensitization to konjac flour in guinea pigs. *Toxicology*. 1997;124:115-124.
79. Dobashi, K. Occupational Asthma in Japan. *Asia Pac.Allergy*. 2012;2:173-180.
80. Verhoef, MERSGZPaMFWHM. Acute histopathological effects of benzalkonium chloride and absorption enhancers on rat nasal epithelium. *Int.J.Pharm*. 1996;141:151-160.
81. Nakazawa, T. Studies on agriculture and asthma. *Jpn.J.Traumatol.Occup.Med*. 1983;32:10-17.
82. Tobacman, JKWRBaZMB. Consumption of carrageenan and other water-soluble polymers used as food additives and incidence of mammary carcinoma. *Medical Hypotheses*. 2001;56(5):589-598.

83. Vido, LFPAIGDaRF. Childhood obesity treatment: Double blinded trial on dietary fibers (glucomannan) versus placebo. *Paediatr.Paedol.* 1993;28:133-136.
84. Pinniger, GJLTaBAJ. Skeletal muscle weakness caused by carrageenan-induced inflammation. *Muscle Nerve.* 2012;46:413-420.
85. Sarithakumari, CHRGLaKGM. Anti-inflammatory and antioxidant potential of alginic acid isolated from the marine algae, *Sargassum wightii* on adjuvant-induced arthritic rats. *Inflammopharmacology.* 2013;21:261-268.
86. Armisen, R. Agar and agarose biotechnological applications. *Hydrobiologia.* 1991;221(1):157-166.
87. Steiner, ABaMWH. Algin in review. *Adv.Chem.Ser.Am.Chem.Soc.* 1954;11:68-82.
88. Soares, JPSJECGOaCETG. Thermal behavior of alginic acid and its sodium salt. *Eclet.Quim.* 2004;29(2):57-63.
89. Jensen, MGPCCKMFGaAA. Review: Efficacy of alginate supplementation in relation to appetite regulation and metabolic risk factors: evidence from animal and human studies. *Obesity Reviews.* 2013;14:129-144.
90. Skjak-Braek, G. Alginates: biosyntheses and some structure-function relationships relevant to biomedical and biotechnological applications. *Biochem.Soc.Trans.* 1992;20:27-33.
91. Beeren, SRaHO. Natures Dendrimer: Characterizing Amylopectin as a Multivalent Host. *Angew.Chem.Int.Ed.* 2013;52:11265 -11268.
92. International Agency for Research on Cancer (IARC). IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Volume 31. Carrageenan. <http://www.iarc.fr>. IARC. Lyon. Last Updated 1983.
93. Norton, ITaGDM. Role of cations in the conformation of iota and kappa carrageenan. *J.Chem.Soc.Farraday Trans.I.* 1983;79:2475-2488.
94. Carareto, NDDMFESPFPAaMAJA. Water activity of aqueous solutions of ethylene oxide-propylene oxideblock polymers and maltodextrins. *Braz.J.Chem.Eng.* 2010;27(1):173-181.
95. Bertoft, EKKaAP. Structure of building blocks in amylopectin. *Carbohydrate Research.* 2012;361:105-113.
96. Saeed, FPIAFMaSMT. Arabinoxylans and arabinogalactans: A comprehensive treatise. *Critical Reviews in Food Science and Nutrition.* 2011;51:467-476.
97. MacGregor, A. W. and Fincher G. B. Carbohydrates of the barley grain. Macgregor, A. W. and Bhatti R. S. In: *Barley: Chemistry and Technology*. St. Paul: American Association of Cereal Chemists; 2014:73-130.
98. Gabrielli, IGPGWGJRKaKL. Separation, characterization and hydrogel-formation of hemicellulose from aspen wood. *Carbohydr.Poly.* 2000;43(4):367-374.

99. Biliaderis, C. G. and Izydorczyk M. S. Arabinoxylans: Technologically and nutritionally functional plant polysaccharides. In: *Functional Food Carbohydrates*. Boca Raton: CRC Press; 2007:249-290.
100. Toole, GAWRHPMLWNKWTRSPRaMENC. The effect of environment on endosperm cell wall development in *Triticum aestivum* during grain filling: An infrared spectroscopy imaging study. *Planta*. 2007;225:1393-1403.
101. Mandalari, GFCBSAISABGaL-CR. Fractionation and characterization of arabinoxylans from brewers' spent grain and wheat bran. *J.Cereal Sci.* 2005;42(2):205-212.
102. Khan, K. and Shewry. P. R. Wheat Chemistry and Technology. 4th ed. St. Paul: American Assn. of Cereal Chemists, 2009.
103. Goellner, EMUJKRaCB. Structure of arabinogalactan from *Larix laricina* and its reactivity with antibodies directed against type-II arabinogalactans. *Carbohydrate Polymers*. 2011;86(4):1739-1744.
104. Elworthy, PHaGTM. The molecular properties of ghatti gum: A naturally occurring polyelectrolyte. *J.Pharm.Pharmacol.* 1963;15:781-793.
105. World Health Organization (WHO). Toxicological evaluation of certain food additives and contaminants. Konjac flour. World Health Organization (WHO) Food Additives Series 32. 1993. <http://www.inchem.org/documents/jecfa/jecmono/v32je10.htm>. Last Updated 1993. Date Accessed 10-1-2014.
106. Nishinari, K. and Gao S. Konjac glucomannan. Chapter: 3. Biliaderis, C. G. and Izydorczyk M. S. In: *Functional Carbohydrates*. 2007:97-125.
107. Carabin, IGaFWG. Evaluation of safety of inulin and oligofructose as dietary fiber. *Regulatory Toxicology and Pharmacology*. 1999;30:268-282.
108. Yoo, MKCHKKTHCYJATSMaCCS. Drug release from xyloglucan beads coated with Eudragit for oral drug delivery. *Arch.Pharm.Res.* 2005;28(6):736-742.
109. Lubrizol Corporation. Sensomer™ CI-590 Polymer (starch hydroxypropyltrimonium chloride). Composition, product specifications, and technical data sheet. <https://www.lubrizol.com/PersonalCare/Products/Sensomer/CI-50.html>. Last Updated 2014. Date Accessed 10-22-2014.
110. Gould, SaSRC. 2-Hydroxy- β -cyclodextrin (HP- β -CD): A toxicology review. *Food and Chemical Toxicology*. 2005;43:1451-1459.
111. Toyoda, KSTUCTKaTM. Carcinogenicity study of β -cyclodextrin in F344 rats. *Food and Chemical Toxicology*. 1997;35:331-336.
112. Alam, NaGPC. Structure of a water-soluble polysaccharide from the seeds of *Cassia angustifolia*. *Planta Medica*. 1986;August(4):308-310.
113. Weiner, MLNDBWRHJFaCSM. A 90-day dietary study on kappa carrageenan with emphasis on the gastrointestinal tract. *Food and Chemical Toxicology*. 2007;45:98-106.

114. Marrs, W. M. The stability of carrageenans to processing. Chapter: 9. P.A. Williams and G.O. Phillips. In: *Gums & Stabilizers for the Food Industry*. Cambridge: The Royal Society of Chemistry; 1998:345-357.
115. The United States Pharmacopeial Convention. Food Chemicals Codex. 8th ed. Rockville: The United States Pharmacopeial Convention, 2012.
116. Te Wierik, GHPEACBACaLCF. Preparation, characterization and pharmaceutical application of linear dextrans. I. Preparation and characterization of amylopectin, metastable amylopectins, and metastable amylose. *Pharmaceutical Research*. 1993;10(9):1274-1279.
117. Food and Drug Administration (FDA). Direct food substances affirmed as generally recognized as safe. Maltodextrin. 21 CFR 184.1444. 2013.
118. Maes, CaDJA. Structural characterization of water extractable and water-unextractable arabinoxylans in wheat bran. *J.Cereal Sci.* 2002;35:315-326.
119. Fincher, G. B. and Stone B. A. Cell walls and their components in cereal grain technology. Pomeranz, Y. In: *Advances in cereal science and technology*. St. Paul: American Association of Cereal Chemists; 1986:207-295.
120. Goesaert, KBVWSCCMGKaDJA. Wheat flour constituents: How they impact bread quality, and how to impact their functionality. *Trends Food Sci. Tech.* 2005;16:12-30.
121. Kaur, LSJaSH. Characterization of gum ghatti (*Anogeissus latifolia*): A structural and rheological approach. *Journal of Food Science*. 2009;74(6):E328-E332.
122. Parry, Jean-Marc. Konjac glucomannan. Chapter: 11. Imeson, A. In: *Food Stabilizers, Thickeners and Gelling Agents*. 2010:198-217.
123. Rahul, RJUSGaMS. Carboxymethyl inulin: A novel flocculant for wastewater treatment. *International Journal of Biological Macromolecules*. 2014;63:1-7.
124. Personal Care Products Council. Information concerning corn starch modified. Method of manufacture of Amaze®. Unpublished data submitted by the Personal Care Products Council on 11-12-2014. 2014. pp.1
125. Personal Care Products Council. Method of manufacture for dextrin and other starch-derived ingredients. Unpublished data submitted by the Personal Care Products Council on 10-31-2014. 2014. pp.1-2.
126. Chiba Flour Milling Co., Ltd. Manufacturing process of Rheopearl (dextrin palmitate and dextrin myristate). Unpublished data submitted by the Personal Care Products Council on 10-10-2014. 2013. pp.1
127. Chiba Flour Milling Co., Ltd. Material safety data sheet on Unifilma HVY (dextrin isostearate). Unpublished data submitted by the Personal Care Products Council on 10-10-2014. 2012. pp.1-7.
128. Chiba Flour Milling Co., Ltd. Manufacturing process of Unifilma HVY (dextrin isostearate). Unpublished data submitted by the Personal Care Products Council on 10-10-2014. 2013. pp.1

129. Chiba Flour Milling Co., Ltd. Material safety data sheet on Rheopearl TT2 (dextrin palmitate/ethylhexanoate). Unpublished data submitted by the Personal Care Products Council on 10-10-2014. 2010. pp.1-6.
130. Bello-Perez, LAS-HLM-DET-VJF. Laboratory scale production of maltodextrins and glucose syrup from banana starch. *Acta Cientifica Venezolana*. 2002;53:44-48.
131. AkzoNobel. Letter from AkzoNobel on the water solubility of sodium hydrolyzed potato starch dodecenylsuccinate. Unpublished data submitted by AkzoNobel on 4-14-2014. 2014. pp.1
132. AkzoNobel. Letter from AkzoNobel on the method of manufacture of sodium hydrolyzed potato starch dodecenylsuccinate. Unpublished data submitted by AkzoNobel on 4-14-2014. 2014. pp.1
133. Anonymous. Chemical structure diagram and descriptive information for production of: starch hydroxypropyltrimethylammonium chloride sodium phthalic acid ester (starch hydroxypropyltrimonium chloride). Unpublished data submitted by the Personal Care Products Council on 11-13-2014. 2014. pp.1
134. Personal Care Products Council. Method of manufacture and impurities. Starch hydroxypropyltrimonium chloride. Unpublished data submitted by the Personal Care Products Council on 10-23-2014. 2014. pp.1
135. Chatjigakis, AKDCCAWaCP. Solubility behavior of β -Cyclodextrin in water/cosolvent mixtures. *Anal.Chem.* 1992;64(14):1632-1634.
136. Personal Care Products Council. Method of manufacture: Algae exopolysaccharides. Unpublished data submitted by the Personal Care Products Council on 11-10-2014. 2014. pp.1
137. Chaubey, MaKVP. Structure of a galactomannan from the seeds of *Cassia angustifolia* Vahl. *Carbohydrate Research*. 2001;332:439-444.
138. Hayashibarta Co., Ltd. TETRUPTM (hydrolyzed starch). Method of manufacture and chemical characterization. Unpublished data submitted by the Personal Care Products Council on 11-20-2014. 2014. pp.1-2.
139. Hayashibarta Co., Ltd. TETRUPTM -H (hydrolyzed starch). Method of manufacture and chemical characterization. Unpublished data submitted by the Personal Care Products Council on 11-20-2014. 2014. pp.1-2.
140. Uno, YOTGYAINMaMT. Molecular weight distribution of carrageenans studied by a combined gel permeation/inductively coupled plasma (GPC/ICP) method. *Food Additives and Contaminants*. 2001;18(9):763-772.
141. Marinalg International. Carageenan (INS 407) and processed eucheuma seaweed (INS 407a) monograph in response to request for data for the 68th meeting of JECFA. www.marinalg.org/wp-content/uploads/2012/09/CGN-PES-monograph-November-2006.pdf. Last Updated 2006.

142. Anonymous. Certificate relative to the use of a cosmetic ingredient: Composition of mixtures containing hydrolyzed furcellaran. Unpublished data submitted by the personal Care Products Council on 10-24-2014. 2014. pp.1
143. Simsek, SaOJB. Enhance hard spring and durum wheat quality and utilization. *Carbohydr.Poly.* 2009;77:87-94.
144. Chiba Flour Milling Co., Ltd. Certificate of composition on Rheopearl MKL2 (dextrin myristate). Unpublished data submitted by the Personal Care Products Council on 10-10-2014. 2014. pp.1
145. Chiba Flour Milling Co., Ltd. Certificate of composition of Rheopearl KL2 (dextrin palmitate). Unpublished data submitted by the Personal Care products Council on 10-10-2014. 2012. pp.1
146. Chiba Flour Milling Co., Ltd. Certificate of composition on Rheopearl TL2 (dextrin palmitate). Submission of unpublished data by the Personal Care Products Council on 10-10-2014. 2012. pp.1
147. Chiba Flour Milling Co., Ltd. Certificate of composition of Rheopearl TT2 (dextrin palmitate/ethylhexanoate). Unpublished data submitted by the Personal Care Products Council on 10-10-2014. 2012. pp.1
148. Chiba Flour Milling Co., Ltd. Certificate of composition of Unifilma HVY (dextrin isostearate). Unpublished data submitted by the Personal Care Products Council on 10-10-2014. 2014. pp.1
149. Analytical Industrial Research Laboratories, Inc. Lab report. PS-111 product (powder). Unpublished data submitted by AkzoNobel on 4-14-2014. 2009. pp.1
150. Chiba Flour Milling Co., Ltd. Certificate of composition on Rheopearl ISK2 and ISL2 (stearoyl inulin). Unpublished data submitted by the Personal Care Products Council on 10-10-2014. 2012. pp.1
151. Kotkoskie, LAWMLFCBKJJGCHCJaFMJ. Acute toxicity studies with konjac flour. *Toxicol.Letters Suppl.* 1992;281
152. Oketani, YIKOCGMKSaKS. Toxicity studies on glucomannan. (1) Acute toxicity study in mice and rats. *Appl.Pharmacol.* 1984;27:127-131.
153. Stanford Research Institute. Study of mutagenic effects of sterculia gum (karaya), FDA No. 71-17. NTIS Report No. PB221823. 1972. pp.1-91.
154. MB Research Laboratories, Inc. Single dose oral toxicity in rats/LD₅₀ in rats. Test article # 73-8050. Unpublished data submitted by AkzoNobel on 4-14-2014. 1996. pp.1-5.
155. Australian Government Department of Health. National Industrial Chemicals Notification and Assessment Scheme (NICNAS) full public report. Potato starch modified. File No. PLC/639. 2006. pp.1-12. Sydney: National Industrial Chemicals Notification and Assessment Scheme.
156. Johannsen, FR. Toxicological profile of carboxymethyl inulin. *Food and Chemical Toxicology.* 2003;41:49-59.

157. Fowler, EFSJGaTW. Histological and ultrastructural changes following carrageenan injection in the mouse. *J.Pathology*. 1980;132:63-79.
158. Kuznetsky, RDTFEaASS. Effects of carrageenan on the mouse hematopoietic system. *Exp.Hemat*. 1980;8(4):465-476.
159. Ahmad, SFZKMA and Abdel-Hamied, HEASMBSAaEaA-AARA. Grape seed proanthocyanidin extract protects against carrageenan-induced lung inflammation in mice through reduction of pro-inflammatory markers and chemokine expressions. *Inflammation*. 2014;37(2):500-511.
160. Elena, TRDEMEEVMaSC. Anti-inflammatory effects of adrenomedullin on acute lung injury induced by carrageenan in mice. *Mediators of Inflammation*. 2012;1-13.
161. Mitsuhashi, TKHaIY. Carrageenan-induced pulmonary emphysema of rabbit. *Tohoku J.Exp.Med*. 1989;157:163-176.
162. Til, HPFJaIHR. Chronic (89-week) feeding study with hydroxypropyl distarch phosphate, starch acetate, lactose and sodium alginate in mice. *Fd.Chem.Toxic*. 1986;24(8):825-834.
163. Francois, IEJALOVWSKRHJEDJBESMGCAaBWF. Safety assessment of a wheat bran extract containing arabinoxylan-oligosaccharides: Mutagenicity, clastogenicity, and 890-day rat-feeding studies. *International Journal of Toxicology*. 2010;29(5):479-495.
164. Maronpot, RRDJMGGDKaHS. Evaluation of 90-day oral rat toxicity studies on the food additive, gum ghatti. *Food and Chemical Toxicology*. 2012;51:215-224.
165. World Health Organization (WHO). Toxicological evaluation of certain food additives. Konjac flour. WHO Food Additive Series 37. 1996. <http://www.inchem.org/documents/jecfa/jecmono/v37je04.htm>. Last Updated 1996. Date Accessed 10-1-2014.
166. Miyoshi, HOTIKOYTMaKH. The effects of cellulose, pectin and konjac intake on growth and digestibility in rats. *Osaka-Shiritsu Daigaku Seikatsulkaoakubu Kiyo*. 1987;35:23-28.
167. Hou, YHZLSZHMWRSaZYZ. Influences of refined konjac meal on the levels of tissue lipids and the absorption of four minerals in rats. *Biomed.Environ.Sci*. 1990;3(3):306-314.
168. Garthoff, JAHSRALBARKCAMKJHaSGJA. Safety evaluation of pectin-derived acidic oligosaccharides (pAOS): Genotoxicity and subchronic studies. *Regulatory Toxicology and Pharmacology*. 2010;57:31-42.
169. Anderson, DMWBAKSAPDW. Transmission electron microscopy of jejunum, ileum, and caecum tissues from rats fed with gums arabic, karaya and tragacanth. *Toxicology*. 1986;41:75-82.
170. Til, HPaBA. Subchronic (13-week) oral toxicity study of γ -cyclodextrin in dogs. *Regulatory Toxicology and Pharmacology*. 1998;27:159-165.
171. Eastwood, MABWGaADMW. The effects of dietary gum karaya (*Sterculia*) in man. *Toxicology Letters*. 1983;17:159-166.

172. Verrett, MJSWFREFAEKaTCA. Toxicity and teratogenicity of food additive chemicals in the developing chicken embryo. *Toxicology and Applied Pharmacology*. 1980;56:265-273.
173. Voorhees, CVBREBRLaSTJ. A developmental test battery for neurobehavioral toxicity in rats: A preliminary analysis using monosodium glutamate, calcium carrageenan, and hydroxyurea. *Toxicology and Applied Pharmacology*. 1979;50:267-282.
174. National Technical Information Service (NTIS). Teratogenic evaluation of *Sterculia* gum. NTIS Report Nos. PB 221/789 and PB 223/818. 1973.
175. Waalkens-Berendsen, DHVFJJaBA. Embryotoxicity and teratogenicity study with γ -cyclodextrin in rats. *Regulatory Toxicology and Pharmacology*. 1998;27:166-171.
176. Waalkens-Berendsen, DHaBA. Embryotoxicity and teratogenicity study with α -cyclodextrin in rats. *Regulatory Toxicology and Pharmacology*. 2004;39:S34-S39.
177. Waalkens-Berendsen, DHSPAEEaBA. Embryotoxicity and teratogenicity study with γ -cyclodextrin in rabbits. *Regulatory Toxicology and Pharmacology*. 1998;27:172-177.
178. Waalkens-Berendsen, DHSPAEEaBA. Embryotoxicity and teratogenicity study with α -cyclodextrin in rabbits. *Regulatory Toxicology and Pharmacology*. 2004;39:S40-S46.
179. Sylianco, CYLBJSEaGE. Non-genotoxic and antigenotoxic activity of PNG carrageenan. *Philippine Journal of Science*. 1993;122(2):139-153.
180. Hobbs, CASCMRDJRLaHS. Evaluation of the genotoxicity of the food additive, gum ghatti. *Food and Chemical Toxicology*. 2012;50:854-860.
181. Harlan Laboratories, Ltd. PS-111 hydrophobically modified starch powder. Reverse mutation assay "Ames test" using *Salmonella typhimurium* and *Escherichia coli*. Project number: 2918/0006. Unpublished data submitted by AkzoNobel on 4-14-2014. 2010. pp.1-32.
182. National Toxicology Program (NTP). Carcinogenesis bioassay of agar (CAS No, 902-18-0) in F344 rats and B6C3F₁ mice (feed study). NTP Technical Report Series No. 230. NIH Publication No. 82-1786. <http://ntp.niehs.nih.gov>. Last Updated 1982.
183. Wilcox, DKHJaBTA. Colonic epithelial cell proliferation in a rat model of nongenotoxin-induced colonic neoplasia. *Laboratory Investigation*. 1992;67(3):405-411.
184. Nilson, HWaWJA. Feeding test with carrageenan. *Food Res*. 1959;24:234-239.
185. Rustia, MSPaPK. Lifespan carcinogenicity tests with native carrageenan in rats and hamsters. *Cancer Lett*. 1980;11:1-10.
186. Watanabe, K, Yakou, S, Takayama, K, Machida, Y, Isowa, K, and Nagai, T. Investigation on rectal absorption of indomethacin from sustained-release hydrogel suppositories prepared with water-soluble dietary fibers, xanthan gum and locust bean gum. *Biol Pharm Bull*. 1993;16(4):391-394.
187. Corpet, DETSaPM. Carrageenan given as a jelly does not initiate, but promotes the growth of aberrant crypt foci in rat colon. *Cancer Letters*. 1997;114:53-55.

188. Tache, SPGMAaCDE. Carrageenan gel and aberrant crypt foci in the colon of conventional and human flora-associated rats. *Nutrition and Cancer*. 2000;37(2):193-198.
189. Hagiwara, AMKNTSMTSAINMIKINaST. Lack of tumor promoting effects of carrageenan on 1,2-dimethylhydrazine-induced colorectal carcinogenesis in male F344 rats. *J.Toxicol.Pathol*. 2001;14:37-43.
190. Moser, ARPHCaDWF. A dominant mutation that predisposes to multiple intestinal neoplasia in the mouse. *Science*. 1990;47:322-324.
191. Su, LKKKWVBPACMARLCEal. Multiple intestinal neoplasia caused by a mutation in the murine homology of the APC gene. *Science*. 1992;256:668-670.
192. Misikangas, MPAPEaMM. Promotion of adenoma growth by dietary inulin is associated with increase in cyclin D1 and decrease in adhesion proteins in Min/+ mice mucosa. *Journal of Nutritional Biochemistry*. 2005;16:402-409.
193. Hijova, ESVSJaHG. Chemopreventive and metabolic effects of inulin on colon cancer development. *J.Vet.Sci*. 2013;14(4):387-393.
194. Taper, HSaRM. Influence of inulin and oligofructose on breast cancer and tumor growth. *J.Nutr*. 1999;129:1488S-1491S.
195. Femia, APSMBWFFIEJADJACCMaCG. Arabinoxylan-oligosaccharides (AXOS) reduce preneoplastic lesions in the colon of rats treated with 1,2-dimethylhydrazine (DMH). *Eur.J.Nutr*. 2010;49:127-132.
196. Cocoa, LLXQTSGGYCFZSaSX. Antitumor and immunomodulatory activity of arabinoxylans: A major constituent of wheat bran. *International Journal of Biological Macromolecules*. 2011;48:160-164.
197. Mizutani, TaMT. Effect of konjac mannan on spontaneous liver tumorigenesis and fecal flora in C3H/He male mice. *Cancer Letters*. 1982;17:27-32.
198. Mizutani, TaMT. Effect of konjac mannan on 1,2-dimethylhydrazine-induced intestinal carcinogenesis in Fisher 344 rats. *Cancer Letters*. 1983;19:1-6.
199. Andoh, ABTaSM. Physiological and anti-inflammatory roles of dietary fiber and butyrate in intestinal functions. *J.Parenter.Enteral Nutr*. 1999;23(5):S70-S73.
200. Waner, TBGCSPiaNA. Investigation of potential oncogenetic effects of β -cyclodextrin in the rat and mouse. *Arch.Toxicol*. 1995;69:631-639.
201. Wakabayashi, KITFYaFY. Induction by degraded carrageenan of colorectal tumors in rats. *Cancer Lett*. 1978;4:171-176.
202. Wakabayashi, K. A study on carcinogenesis of degraded carrageenan, a polysaccharide-sulfate, derived from seaweeds (Jpn.). *Juntendo Med.J*. 1981;27:159-171.
203. Oohashi, YITWKaKN. A study on carcinogenesis induced by degraded carrageenan arising from squamous metaplasia of the rat colorectum. *Cancer Lett*. 1981;14:267-272.

204. Unilever Research U.S. Primary dermal irritancy test in rabbits on national 73-8050. Unpublished data submitted by AkzoNobel on 4-14-2014. 1996. pp.1
205. Kawahara, DOHKHNMSTaTT. Further epidemiologic study of occupational contact dermatitis in the dental clinic. *Contact Dermatitis*. 1993;28:114-115.
206. Thomas J. Stephens & Associates, Inc. Human repeat insult patch test of an eye gel containing 2.45% maltodextrin. Unpublished data submitted by the Personal Care Products Council on 6-26-2014. 1997. pp.1-12.
207. Essex Testing Clinic, Inc. Clinical safety evaluation. Repeated insult patch test of a rinse-off facial product containing 42.6919% dextrin. Unpublished data submitted by the Personal Care Products Council on 10-30-2014. 2005. pp.1-11.
208. Essex Testing Clinic, Inc. Clinical safety evaluation. Repeated insult patch test of a leave-on facial product containing 0.3% dextrin myristate. Unpublished data submitted by the Personal Care Products Council on 10-30-2014. 2009. pp.1-10.
209. Consumer Product Testing Co. Repeated insult patch test of 15% hydroxypropyltrimonium hydrolyzed corn starch. Unpublished data submitted by the Personal Care Products Council on 11-21-2014. 2005. pp.1-9.
210. Hill Top Research, Inc. Revised report for a 21-day cumulative irritation study in humans. Test article # 73-8050. Project No. 96-4063-74. Unpublished data submitted by AkzoNobel on 4-14-2014. 1996. pp.1-7.
211. Harrison Research laboratories, Inc. Repeated insult patch test (RIPT). Final report. Cleanser containing 10 wt% sodium hydrolyzed potato starch dodecenylsuccinate. Unpublished data submitted by AkzoNobel on 10-23-2014. 2009. pp.1-17.
212. Anonymous. Summary of a 50 human subject repeat insult patch test skin irritation/sensitization test of 1% algae exopolysaccharides. Unpublished data submitted by the Personal Care Products Council on 11-10-2014. 2009. pp.1-2.
213. Consumer Product Testing Co. The MatTek Corporation EpiDerm™ skin model *in vitro* toxicity testing system: Hydroxypropyltrimonium hydrolyzed corn starch. Unpublished data submitted by the Personal Care Products Council on 11-21-2014. 2004. pp.1-5.

ADOPTED: 24 January 2017

doi: 10.2903/j.efsa.2017.4699

Safety of the proposed amendment of the specifications for microcrystalline cellulose (E 460(i)) as a food additive

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS)

Abstract

Following a request from the European Commission, the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) provided a scientific opinion regarding the safety of an amendment of the specifications for the food additive microcrystalline cellulose (E 460(i)). The scope of the current application was to amend the solubility of the food additive to 'practically insoluble or insoluble in sodium hydroxide solution'. Currently, the existing EU specifications describe microcrystalline cellulose as 'slightly soluble in sodium hydroxide'. In support of the request, the applicant carried out solubility tests on four batches of microcrystalline cellulose (E 460(i)). Because the concentration of sodium hydroxide solution to be used for the solubility tests is not indicated in the applicable JECFA testing procedures, the applicant performed the test using a concentration (50 g/L) as specified in the European Pharmacopeia. The tests indicated that the quantity of sodium hydroxide solution required to dissolve 1 g of the sample is more than 10,000 mL. The Panel noted that no analytical measurements of the solubility in a range of concentrations of sodium hydroxide solution were submitted in the application dossier and that, according to published information, the solubility of microcrystalline cellulose depends on the concentration of sodium hydroxide solution. The Panel also noted that in the current EU specifications for microcrystalline cellulose (E 460(i)) the concentration of sodium hydroxide solution to be used for testing solubility is not given. Therefore, the Panel concluded that the amendment to the specifications as regards the solubility of microcrystalline cellulose (E 460(i)) proposed by the applicant would not give rise to a safety concern. However, the Panel recommended that the concentration of sodium hydroxide solution to be used in the solubility test should be indicated in the EU specifications.

© 2017 European Food Safety Authority. *EFSA Journal* published by John Wiley and Sons Ltd on behalf of European Food Safety Authority.

Keywords: food additive, microcrystalline cellulose, E 460(i), changes in specifications, solubility

Requestor: European Commission

Question number: EFSA-Q-2016-00199

Correspondence: fip@efsa.europa.eu

Panel members: Fernando Aguilar, Riccardo Crebelli, Alessandro Di Domenico, Birgit Dusemund, Maria Jose Frutos, Pierre Galtier, David Gott, Ursula Gundert-Remy, Claude Lambré, Jean-Charles Leblanc, Oliver Lindtner, Peter Moldeus, Alicja Mortensen, Pasquale Mosesso, Dominique Parent-Massin, Agneta Oskarsson, Ivan Stankovic, Ine Waalkens-Berendsen, Rudolf Antonius Woutersen, Matthew Wright and Maged Younes.

Acknowledgements: The Panel wishes to thank the members of the Working Group on Applications: Maria Jose Frutos, David Gott, Lieve Herman, Claude Lambré, Jean-Charles Leblanc, Peter Moldeus, Alicja Mortensen, Ivan Stankovic, Paul Tobback, Ine Waalkens-Berendsen, Rudolf Antonius Woutersen and Matthew Wright for the preparatory work on this scientific output and EFSA staff members: Fabiola Pizzo and Camilla Smeraldi for the support provided to this scientific output.

Suggested citation: EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), 2017. Safety of the proposed amendment of the specifications for microcrystalline cellulose (E 460(i)) as a food additive. *EFSA Journal* 2017;15(2):4699, 7 pp. doi:10.2903/j.efsa.2017.4699

ISSN: 1831-4732

© 2017 European Food Safety Authority. *EFSA Journal* published by John Wiley and Sons Ltd on behalf of European Food Safety Authority.

This is an open access article under the terms of the [Creative Commons Attribution-NoDerivs](https://creativecommons.org/licenses/by-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited and no modifications or adaptations are made.



The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.



Table of contents

Abstract.....	1
1. Introduction.....	4
1.1. Background and Terms of Reference as provided by the requestor.....	4
1.1.1. Background	4
1.1.2. Terms of Reference	4
1.2. Interpretation of the Terms of Reference.....	4
1.2.1. Information on existing evaluations and authorisations.....	4
2. Data and methodologies	4
2.1. Data.....	4
2.2. Methodologies.....	5
3. Assessment.....	5
3.1. Technical data.....	5
3.1.1. Specifications	5
3.1.2. Manufacturing process.....	6
4. Discussion	6
5. Conclusions.....	7
6. Recommendations.....	7
Documentation provided to EFSA	7
References.....	7
Abbreviations	7

1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

1.1.1. Background

The use of food additives is regulated under the European Parliament and Council Regulation (EC) No 1333/2008¹ on food additives. Only food additives that are included in the Union list, in particular in Annex II to that Regulation, may be placed on the market and used in foods under conditions of use specified therein. Moreover, food additives shall comply with the specifications as referred to in Article 14 of Regulation and laid down in Commission Regulation (EU) No 231/2012².

Microcrystalline cellulose (E 460(i)) is an authorised food additive in the European Union (EU) for use in several food categories and specifications have been adopted for it. Presently, those specifications stipulate for the parameter 'Solubility' that the food additive is 'Insoluble in water, ethanol, ether and dilute mineral acids. Slightly soluble in sodium hydroxide solution'.

The European Commission received a request vis-à-vis an amendment of the present specifications of microcrystalline cellulose (E 460(i)) for that parameter 'Solubility' as follows: 'Insoluble in water, ethanol, ether and dilute mineral acids. Practically insoluble or insoluble in sodium hydroxide solution'.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority (EFSA) to provide a scientific opinion as regards a proposed amendment of the specifications of the food additive microcrystalline cellulose (E 460(i)) in accordance with Regulation (EC) No 1331/2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings.

1.2. Interpretation of the Terms of Reference

In order to address the safety of this proposed change in specifications, the Panel decided that an evaluation of the possible impact of this proposed change on the characteristic of the product and on its safety profile would be adequate.

The Panel considered that this change was not due to a modification in the manufacturing process of microcrystalline cellulose (E 460 (i)).

The safety of microcrystalline cellulose (E 460(i)) as food additive³ will be considered under the re-evaluation programme for food additives already permitted in the EU before 20 January 2009, as envisaged by Commission Regulation (EU) No 257/2010. Meanwhile, the Panel considered that the available toxicological assessment of microcrystalline cellulose would remain valid and in principle there would be no need to reconsider this to address the Terms of Reference.

1.2.1. Information on existing evaluations and authorisations

Currently, microcrystalline cellulose (E 460(i)) is an authorised food additive in the EU at quantum satis (QS), in almost all authorised food categories. It is also included in Group I of food additives authorised at QS.

The safety of microcrystalline cellulose (E 460(i)) as a food additive is currently being re-evaluated by EFSA.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier requesting changes to existing specifications. The application was supported by data related to the solubility of the food additive microcrystalline cellulose (E 460(i)) (Documentation provided to EFSA n. 1).

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 Regulation (EC) 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.

³ EFSA Register of Questions: <http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2011-00545>

2.2. Methodologies

The current 'Guidance for submission for food additive evaluations' (EFSA ANS Panel, 2012) has been followed by the Panel for evaluating the proposed change in specifications, with regard to the solubility.

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

The interpretation of the descriptive term for approximate solubility 'practically insoluble or insoluble' in the Identification Tests in JECFA specifications means that more than 10,000 parts of solvent is required for 1 part of solute (JECFA, 2006).

3. Assessment

3.1. Technical data

3.1.1. Specifications

The proposed amendment by the applicant to the existing EU specifications for the food additive microcrystalline cellulose (E 460(i)), is given in Table 1.

Table 1: Existing EU specifications for food additive microcrystalline cellulose (E 460(i)) according to Commission Regulation (EU) No 231/2012 and proposal for amendment as requested by the applicant

	Specifications according to Commission Regulation (EU) No 231/2012	Requested amendment by the applicant
Synonyms	Cellulose gel	None
Definition	Microcrystalline cellulose is purified, partially depolymerised cellulose prepared by treating alpha-cellulose, obtained as a pulp from strains of fibrous plant material, with mineral acids. The degree of polymerisation is typically less than 400	None
Einecs	232-674-9	None
Chemical name	Cellulose	None
Chemical formula	(C ₆ H ₁₀ O ₅) _n	None
Molecular weight	About 36,000	None
Assay	Not less than 97% calculated as cellulose on an anhydrous basis	None
Particle size	Not less than 5 µm (not more than 10% of particles of less than 5 µm)	None
Description	A fine white or almost white odourless powder	None
Identification		
Solubility	Insoluble in water, ethanol, ether and dilute mineral acids. Slightly soluble in sodium hydroxide solution	Insoluble in water, ethanol, ether and dilute mineral acids. Practically insoluble or insoluble in sodium hydroxide solution
Colour reaction	To 1 mg of the sample, add 1 mL of phosphoric acid and heat on a water bath for 30 min. Add 4 mL of a 1 in 4 solution of pyrocatechol in phosphoric acid and heat for 30 min. A red colour is produced	None
Infrared absorption spectroscopy	To be identified	None

	Specifications according to Commission Regulation (EU) No 231/20102	Requested amendment by the applicant
Suspension test	Mix 30 g of the sample with 270 mL of water in a high-speed (12,000 rpm) power blender for 5 min. The resultant mixture will be either a free-flowing suspension or a heavy, lumpy suspension which flows poorly, if at all, settles only slightly and contains many trapped air bubbles. If a free-flowing suspension is obtained, transfer 100 mL into a 100-mL graduated cylinder and allow to stand for 1 h. The solids settles and a supernatant liquid appears	None
pH	The pH of the supernatant liquid is between 5.0 and 7.5) 10% suspension in water)	None
Purity		
Loss on drying	Not more than 7% (105°C, 3 h)	None
Water-soluble matter	Not more than 0.24%	None
Sulphated ash	Not more than 0.5% (800 ± 25°C)	None
Starch	Not detectable To 20 mL of the dispersion obtained in identification, suspension test, add a few drops of iodine solution and mix. No purplish to blue or blue colour should be produced	None
Carboxyl groups	Not more than 1%	None
Arsenic	Not more than 3 mg/kg	None
Lead	Not more than 2 mg/kg	None
Mercury	Not more than 1 mg/kg	None
Cadmium	Not more than 1 mg/kg	None

The applicant provided data related to solubility of microcrystalline cellulose in sodium hydroxide solution as part of the dossier. The tests were performed on four different batches and in compliance with the JECFA testing procedures for solubility (JECFA, 2006). Because the concentration of sodium hydroxide solution to be used for the solubility tests is not indicated in the JECFA testing procedures, the applicant performed the test using a concentration (50 g NaOH/L) as specified in the European Pharmacopeia (Ph.Eur. 8.0, 2014).

The analyses were performed as follows: four individual quantities of microcrystalline cellulose, i.e. 0.0089, 0.0088, 0.0089 and 0.0088 g, respectively, were mixed with 100.0 mL of sodium hydroxide solution (concentration: 50 g NaOH/L), corresponding to 1 g of the sample mixed in 10,000 mL of sodium hydroxide solution.

3.1.2. Manufacturing process

The Panel noted that the applicant has submitted information on the manufacturing process used to produce microcrystalline cellulose which is consistent with the manufacturing process in the definition of the food additive according to Regulation (EU) No 231/2012.

4. Discussion

No analytical measurements of the solubility in a range of sodium hydroxide solution were submitted in the application dossier.

The Panel noted that according to published information (Kuo and Hong, 2005), the solubility of microcrystalline cellulose depends on the concentration of sodium hydroxide solution.

The Panel considered that this proposed change in the definition of solubility was not due to a modification in the manufacturing process of microcrystalline cellulose (E 460 (i)).

In the current EU specifications for microcrystalline cellulose (E 460(i)) the concentration of sodium hydroxide solution to be used for testing solubility is not given.

5. Conclusions

Following the request by the European Commission, the Panel considered that the change in the specifications as regards the solubility of microcrystalline cellulose (E 460(i)) proposed by the applicant would not give rise to a safety concern.

6. Recommendations

The Panel recommended that the concentration of sodium hydroxide solution to be used for the solubility test should be indicated in the EU specifications.

Documentation provided to EFSA

- 1) Dossier 'Application for a modification of the specifications of an already authorised food additive – MICROCRYSTALLINE CELLULOSE, CELLULOSE GEL, E 460 (i)'. Submitted by ASAHI KASEI Chemicals Corporation on 26 January 2016.

References

- EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), 2012. Guidance for submission for food additives evaluations. EFSA Journal 2012;10(7):2760, 60 pp. doi:10.2903/j.efsa.2006.391a.
- EFSA SC (Scientific Committee), 2009. Guidance of the Scientific Committee on transparency in the scientific aspects of risk assessment carried out by EFSA. Part 2: general principles. EFSA Journal 2009;7(5):1051, 22 pp. doi:10.2903/j.efsa.2009.1051.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2006. Analytical methods, test procedures and laboratory solutions used by and referenced in the food additive specifications. Combined Compendium of Food Additive Specifications, Vol. 4. Food and Agriculture Organization of the United Nations, Rome, 41 pp.
- Kuo YN and Hong J, 2005. Investigation of solubility of microcrystalline cellulose in aqueous NaOH. Polymers for Advanced Technologies, 16, 425–428.
- Ph.Eur. 8.0, 2014. Microcrystalline Cellulose. European Pharmacopoeia, pp. 1824–1828.

Abbreviations

ANS Panel	EFSA Panel on Food Additives and Nutrient Sources added to Food
JECFA	Joint FAO/WHO Expert Committee on Food Additives
QS	quantum satis



COMPENDIUM OF FOOD ADDITIVE SPECIFICATIONS

Joint FAO/WHO Expert Committee on Food Additives

86th Meeting 2018



Food and Agriculture
Organization of the
United Nations



World Health
Organization

**COMPENDIUM
OF FOOD ADDITIVE
SPECIFICATIONS**

Joint FAO/WHO Expert Committee on Food Additives

**86th Meeting
Geneva, 12 – 21 June 2018**

Required citation:

FAO and WHO. 2018. Compendium of Food Additive Specifications. Joint FAO/WHO Expert Committee on Food Additives (JECFA), 86th Meeting June 2018. FAO JECFA Monographs 22. Rome. 167 pp.
Licence: CC BY-NC-SA 3.0 IGO.

The designations employed and the presentation of material in this information product do not imply the expression of any opinion whatsoever on the part of the Food and Agriculture Organization of the United Nations (FAO) or the World Health Organization (WHO) concerning the legal or development status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. The mention of specific companies or products of manufacturers, whether or not these have been patented, does not imply that these have been endorsed or recommended by FAO or WHO in preference to others of a similar nature that are not mentioned.

The views expressed in this information product are those of the author(s) and do not necessarily reflect the views or policies of FAO or WHO.

ISBN 978-92-5-131100-4 (FAO)

© FAO and WHO, 2018



Some rights reserved. This work is made available under the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 IGO licence (CC BY-NC-SA 3.0 IGO; <https://creativecommons.org/licenses/by-nc-sa/3.0/igo/>).

Under the terms of this licence, this work may be copied, redistributed and adapted for non-commercial purposes, provided that the work is appropriately cited. In any use of this work, there should be no suggestion that FAO or WHO endorses any specific organization, products or services. The use of the FAO or WHO logo is not permitted. If the work is adapted, then it must be licensed under the same or equivalent Creative Commons license. If a translation of this work is created, it must include the following disclaimer along with the required citation: "This translation was not created by the Food and Agriculture Organization of the United Nations (FAO) or the World Health Organization (WHO). Neither FAO nor WHO is responsible for the content or accuracy of this translation. The original [Language] edition shall be the authoritative edition.

Disputes arising under the licence that cannot be settled amicably will be resolved by mediation and arbitration as described in Article 8 of the licence except as otherwise provided herein. The applicable mediation rules will be the mediation rules of the World Intellectual Property Organization <http://www.wipo.int/amc/en/mediation/rules> and any arbitration will be conducted in accordance with the Arbitration Rules of the United Nations Commission on International Trade Law (UNCITRAL).

Users wishing to reuse material from this work that is attributed to a third party, such as tables, figures or images, are responsible for determining whether permission is needed for that reuse and for obtaining permission from the copyright holder. The risk of claims resulting from infringement of any third-party-owned component in the work rests solely with the user.

FAO information products are available on the FAO website (www.fao.org/publications) and can be purchased through publications-sales@fao.org. Requests for commercial use should be submitted via: www.fao.org/contact-us/licence-request. Queries regarding rights and licensing should be submitted to: copyright@fao.org.

SPECIAL NOTE

While the greatest care has been exercised in the preparation of this information, FAO expressly disclaims any liability to users of these procedures for consequential damages of any kind arising out of, or connected with, their use.

Contents

LIST OF PARTICIPANTS.....	VII
INTRODUCTION.....	IX
SPECIFICATIONS FOR CERTAIN FOOD ADDITIVES.....	1
ANIONIC METHACRYLATE COPOLYMER.....	3
BASIC METHACRYLATE COPOLYMER.....	9
CASSIA GUM	15
CITRIC AND FATTY ACID ESTERS OF GLYCEROL (TENTATIVE).....	23
ERYTHROSINE.....	27
GLYCEROL ESTER OF WOOD ROSIN	35
INDIGOTINE.....	41
LUTEIN FROM TAGETES ERECTA.....	47
MODIFIED STARCHES	53
NEUTRAL METHACRYLATE COPOLYMER (TENTATIVE)	87
SPIRULINA EXTRACT (TENTATIVE).....	93
SPECIFICATIONS FOR CERTAIN FLAVOURING AGENTS	99
GROUP 1: MISCELLANEOUS NITROGEN-CONTAINING SUBSTANCES.....	100
GROUP 2: SATURATED ALIPHATIC ACYCLIC BRANCHED-CHAIN.....	102
PRIMARY ALCOHOLS, ALDEHYDES, AND ACIDS.....	102
GROUP 3: LINEAR AND BRANCHED-CHAIN ALIPHATIC, UNSATURATED, UNCONJUGATED ALCOHOLS, ALDEHYDES, ACIDS, AND RELATED ESTERS	103
GROUP 4: CARVONE AND STRUCTURALLY RELATED SUBSTANCES	104
GROUP 5: MENTHOL AND STRUCTURALLY RELATED SUBSTANCES.....	106
GROUP 6: MALTOL AND RELATED SUBSTANCES.....	110
GROUP 7: ALICYCLIC PRIMARY ALCOHOLS, ALDEHYDES, ACIDS	111
AND RELATED ESTERS.....	111
GROUP 8: FURAN SUBSTITUTED ALIPHATIC HYDROCARBONS, ALCOHOLS, ALDEHYDES, KETONES, CARBOXYLIC ACIDS AND RELATED ESTERS, ...	113
SULFIDES, DISULFIDES AND ETHERS.....	113
REVISIONS TO EXISTING FLAVOUR SPECIFICATIONS.....	126
SPECTRA OF CERTAIN FLAVOURING AGENTS	127
CORRIGENDUM.....	137
ANNEX I: SUMMARY OF RECOMMENDATIONS FROM THE 86 th JECFA.....	141
ANNEX 2. GENERAL INFORMATION	153
ANNEX 3. FUTURE WORK AND RECOMMENDATIONS.....	155
SPECIFIC FOOD ADDITIVES (OTHER THAN FLAVOURING AGENTS).....	155
FLAVOURING AGENTS	157

LIST OF PARTICIPANTS

JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES, 86th MEETING Geneva, 12 – 21 June 2018

Members

- Dr S. Barlow, Brighton, East Sussex, England, United Kingdom
- Dr J. Bend, Department of Pathology and Laboratory Medicine, Schulich Medicine & Dentistry, Western University, London, Ontario, Canada
- Dr D. Benford, Cheddington, London, England, United Kingdom
- Dr R. Cantrill, Halifax, Nova Scotia, Canada (Vice-Chairperson)
- Dr E. Dessipri, General Chemical State Laboratory, Athens, Greece, currently European Directorate for the Quality of Medicines and Health Care, Strasbourg, France
- Dr D. Folmer, Office of Food Additive Safety, Center for Food Safety and Applied Nutrition, United States Food and Drug Administration, College Park, Maryland, USA (Joint Rapporteur)
- Ms T. Hambridge, Food Standards Australia New Zealand (FSANZ), Kingston, ACT, Australia
- Dr A. Mattia, Office of Food Additive Safety, Center for Food Safety and Applied Nutrition, United States Food and Drug Administration, College Park, Maryland, USA (Chairperson)
- Dr U. Mueller, Australian Pesticides and Veterinary Medicines Authority (APVMA), Symonston, Australian Capital Territory (ACT), Australia (Joint Rapporteur)
- Dr O.E. Orisakwe, University of Port Harcourt, Choba, Port Harcourt, Rivers State, Nigeria
- Dr J. Schlatter, Zurich, Switzerland
- Dr J. Smith, Bio|Food|Tech, Charlottetown, Prince Edward Island, Canada
- Dr J.R. Srinivasan, Division of Biotech and GRAS Notice Review, Office of Food Additive Safety, Center for Food Safety and Applied Nutrition, United States Food and Drug Administration, College Park, Maryland, USA
- Dr M. Veerabhadra Rao, Hyderabad, India

Secretariat

- Dr J.H. Andersen, National Food Institute, Technical University of Denmark, Lyngby, Denmark (FAO Expert)
- Dr J.N. Barrows, Office of Cosmetics and Colors, Center for Food Safety and Applied Nutrition, United States Food and Drug Administration, College Park, Maryland, USA (FAO Expert)
- Dr C. Béchaux, ANSES, Maisons-Alfort, France (WHO Temporary Adviser)
- Dr M. DiNovi, Office of Food Additive Safety, Center for Food Safety and Applied Nutrition, United States Food and Drug Administration, College Park, Maryland, USA (WHO Temporary Adviser)
- Dr B. Fallico, Food Science and Technology Unit, University of Catania, Catania, Italy (FAO Expert)

- Mr Y. Fan, China National Center for Food Safety Risk Assessment, Beijing, China (CCFA Chairperson)
- Dr M.J.F. Fernandez, Universidad Miguel Hernández, Alicante, Spain (FAO Expert)
- Dr B. Fields, Food Standards Australia New Zealand, Barton, Australian Capital Territory (ACT), Australia (WHO Temporary Adviser)
- Ms F. Hill, Food Standards Agency, London, United Kingdom (WHO Temporary Adviser)
- Dr S.M.F. Jeurissen, Centre for Nutrition, Prevention and Health Services, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands (WHO Temporary Adviser)
- Dr X. Jia, Laboratory of Toxicology, China National Center for Food Safety Risk Assessment, Beijing, China (WHO Temporary Adviser)
- Dr S. Kim, Department of Food Safety and Zoonoses, World Health Organization, Geneva, Switzerland (WHO Secretariat)
- Ms K. Laurvick, United States Pharmacopeial Convention, Rockville, Maryland, USA (FAO Expert)
- Dr M. Lipp, Agriculture and Consumer Protection Department, Food and Agriculture Organization of the United Nations, Rome, Italy (FAO Joint Secretary)
- Dr P. Mosesso, Department of Ecological and Biological Sciences, Università degli Studi della Tuscia, Viterbo, Italy (WHO Temporary Adviser)
- Ms J. Odrowaz, Toronto, Ontario, Canada (WHO Technical Editor)
- Mr K. Petersen, Department of Food Safety and Zoonoses, World Health Organization, Geneva, Switzerland (WHO Secretariat)
- Dr L. Rosenfeld, Division of Petition Review, Office of Food Additive Safety, Center for Food Safety and Applied Nutrition, United States Food and Drug Administration, College Park, Maryland, USA (WHO Temporary Adviser)
- Dr J. Rotstein, Pre-Market Toxicology Assessment Section, Chemical Health Hazard Assessment Division, Bureau Chemical Safety, Food Directorate, Health Products and Food Branch, Health Canada, Ottawa, Ontario, Canada (WHO Temporary Adviser)
- Dr N. Sugimoto, Division of Food Additives, National Institute of Health Sciences (NIHS), Kanagawa, Japan (FAO Expert)
- Dr S. Takasu, Division of Pathology, Biological Safety Research Center, National Institute of Health Sciences, Kawasaki-shi, Kanagawa, Japan (WHO Temporary Adviser)
- Dr A. Tritscher, Department of Food Safety and Zoonoses, World Health Organization, Geneva, Switzerland (WHO Joint Secretary)
- Dr T. Umemura, Faculty of Animal Science Technology, Yamazaki Gakuen University, Tokyo, Japan (WHO Temporary Adviser)
- Dr X. Yang, Food Safety and Health Research Center, Southern Medical University, Guangzhou, Guangdong Province, China (WHO Temporary Adviser)
- Ms L. Zhang, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization of the United Nations, Rome, Italy (Codex Secretariat)

INTRODUCTION

This volume of FAO JECFA Monographs contains specifications of identity and purity prepared at the 86th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), held in Geneva, 12 – 21 June 2018. The specifications monographs are one of the outputs of JECFA's risk assessment of food additives, and should be read in conjunction with the safety evaluation, reference to which is made in the section at the head of each specifications monograph. Further information on the meeting discussions can be found in the summary report of the meeting (see Annex 1), and in the full report which will be published in the WHO Technical Report series. Toxicological monographs of the substances considered at the meeting will be published in the WHO Food Additive Series.

Specifications monographs prepared by JECFA up to the 65th meeting, other than specifications for flavouring agents, have been published in consolidated form in the Combined Compendium of Food Additive Specifications which is the first publication in the series FAO JECFA Monographs. This publication consists of four volumes, the first three of which contain the specifications monographs on the identity and purity of the food additives and the fourth volume contains the analytical methods, test procedures and laboratory solutions required and referenced in the specifications monographs. FAO maintains an on-line searchable database of all JECFA specifications monographs from the FAO JECFA Monographs, which is available at: <http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/>. The specifications for flavourings evaluated by JECFA, and previously published in FAO Food and Nutrition Paper 52 and subsequent Addenda, are included in a database for flavourings specifications. All specifications for flavourings that have been evaluated by JECFA since its 44th meeting, including the 79th meeting, are available in the online searchable database at the JECFA website at FAO: <http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-flav/en/>. The databases have query pages and background information in English, French, Spanish, Arabic and Chinese. Information about analytical methods referred to in the specifications is available in the Combined Compendium of Food Additive Specifications (Volume 4), which can be accessed from the query pages.

An account of the purpose and function of specifications of identity and purity, the role of JECFA specifications in the Codex system, the link between specifications and methods of analysis, and the format of specifications, are set out in the Introduction to the Combined Compendium, which is available in shortened format online on the query page, which could be consulted for further information on the role of specifications in the risk assessment of additives.

Chemical and Technical Assessments (CTAs) for some of the food additives have been prepared as background documentation for the meeting. These documents are available online at: <http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/technical-assessments/en/>.

Contact and Feedback

More information on the work of the Committee is available from the FAO homepage of JECFA at: <http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/en/>. Readers are invited to address comments and questions on this publication and other topics related to the work of JECFA to:

jecfa@fao.org

SPECIFICATIONS FOR CERTAIN FOOD ADDITIVES

New and revised specifications

New (N) or revised (R) specifications monographs were prepared for 11 food additives and these are presented in this publication.

Anionic methacrylate copolymer (AMC) (N, T)

Basic methacrylate copolymer (BMC) (N)

Cassia gum (R)

Citric and fatty acid esters of glycerol (R, T)

Erythrosine (R)

Glycerol ester of wood rosin (R)

Indigotine (R)

Lutein (R)

Modified starches¹ (R, T)

Neutral methacrylate copolymer (NMC) (N, T)

Spirulina extract (N)

In the specifications monographs that have been assigned a tentative status (T), there is information on the outstanding data and a timeline by which this information should be submitted to the FAO JECFA Secretariat.

¹ Applying to all 16 modified starches: INS 1400, 1401, 1402, 1403, 1404, 1405, 1410, 1412, 1413, 1414, 1420, 1422, 1440, 1442, 1450, 1451

ANIONIC METHACRYLATE COPOLYMER

New specifications prepared at the 86th JECFA (2018) and published in FAO JECFA Monographs 22 (2018). No ADI was established at the 86th JECFA (2018).

SYNONYMS E 1207, INS No. 1207, acrylates copolymers, Methyl acrylate, methyl methacrylate, methacrylic acid polymer; methacrylic acid, polymer with methyl acrylate and methyl methacrylate

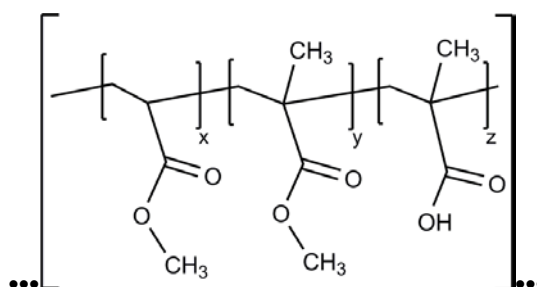
DEFINITION Anionic methacrylate copolymer is a copolymer comprised of monomers, methyl acrylate, methyl methacrylate, and methacrylic acid in the molar ratio of 7:3:1. The copolymer is manufactured by emulsion polymerization of the monomers with water soluble radical initiators. The product is purified by water vapour distillation and filtration to remove residual monomers, excess water, other volatile low-molecular weight substances and coagulum. The copolymer is standardized as a 30% aqueous dispersion. The copolymer dispersion may contain residual monomers (methyl acrylate, methyl methacrylate, and methacrylic acid). Anionic methacrylate copolymer is used as a coating and glazing agent for food supplements and products for special medical purposes.

Chemical name Poly (methyl acrylate-co-methylmethacrylate-co-methacrylic acid) 7:3:1

C.A.S. number 26936-24-3

Chemical formula $\text{Poly}[(\text{CH}_2\text{:CHCO}_2\text{CH}_3)\text{-co-}(\text{CH}_2\text{:C}(\text{CH}_3)\text{CO}_2\text{CH}_3)\text{-co-}(\text{CH}_2\text{:C}(\text{CH}_3)\text{COOH})]$

Structural formula



The above formula is provided for illustrative purposes; in this copolymer no definitive structural unit can be defined.

Formula weight 280,000 (weight-average), 77,000 (number-average)

Assay 9.2 – 12.3 % methacrylic acid units on the dried basis

See description under Tests

DESCRIPTION Commercial form (30% aqueous dispersion) is a low viscosity, milky-white liquid.

FUNCTIONAL USES Coating agent, glazing agent.

CHARACTERISTICS

IDENTIFICATION

Viscosity (Vol. 4) Not more than 20 mPa•s

Determine viscosity using Brookfield viscometer at 20° and 30 rpm using UL adapter.

pH (Vol 4) 2.0 – 3.5

Infrared absorption (Vol. 4) The infrared absorption spectrum of a dry film of sample corresponds to the infrared spectrum in the Appendix.

Apply one drop of sample to a glass plate, cover with a water-resistant crystal disc (AgCl, KRS 5), press lightly, remove the crystal disc and dry for about 15 minutes at 60°.

PURITY

Loss on drying (Vol. 4) 68.5 – 71.5% (110°, 5 h)

Sulfated ash (Vol. 4) Not more than 0.2%

Test 5 g of the sample (Method I)

Methanol (Vol. 4) Not more than 1,000 mg/kg

Residual monomers

Methyl acrylate: Not more than 1 mg/kg

Methyl methacrylate: Not more than 3 mg/kg

Methacrylic acid: Not more than 1 mg/kg

See description under TESTS

Lead (Vol. 4) Not more than 1.0 mg/kg in the dispersion

Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).

<u>Microbiological criteria</u> (Vol. 4)	Total plate count: Less than 1,000 cfu/g Yeast and moulds: Less than 100 cfu/g Coliforms: Negative in 10 g

TESTS

IDENTIFICATION TESTS

<u>Residual monomers</u>	Determined by liquid chromatography (Vol. 4)

Standards and Reagents:

- Acetonitrile: HPLC grade with UV absorption: $A_{\max}$ of 1% at 190 nm
- Acetone, methanol, isobutanol and deionized water: HPLC grade
- Phosphoric acid solution (pH 2): Adjust phosphoric acid (85 %) with an appropriate volume of deionized water to pH 2.
- Standards: methyl acrylate, methyl methacrylate and methacrylic acid (>99%)

Preparation of mixed standard solutions:

Stock mixed standard solution:

Pipette 5 ml of isobutanol into a 50 ml volumetric flask. Accurately weigh approximately 10 mg of methyl acrylate, 12 mg of methyl methacrylate and 11 mg of methacrylic acid, add to isobutanol and dilute to volume with acetone.

Intermediate mixed standard solution-1:

Dilute 5.0 ml of stock mixed standard solution to 50 ml with acetone in a volumetric flask.

Intermediate mixed standard solution-2: Dilute 20.0 ml of intermediate mixed standard solution-1 to 50 ml with acetone in a volumetric flask.

Working mixed standard solution:

Dilute 5 ml of Intermediate mixed standard solution-2 to 25 ml with methanol:phosphoric acid-pH 2 (70:30) in a volumetric flask.

Preparation of sample solution:

Accurately weigh approximately 11 g of sample, dissolve in acetone and dilute to 50 ml in a volumetric flask. Add 5.0 ml of

the solution dropwise (precipitation of the polymer should be slow to avoid entrapment of monomer in the precipitate) to 20 ml methanol and phosphoric acid- pH 2 (70:30 v/v). Centrifuge until the supernatant is clear and use the supernatant as the sample solution.

Procedure:

- Use an HPLC with diode array or UV detector at 200 nm
- Column: Octadecylsilane chemically bonded to porous silica (125 cm x 4.6 mm i.d. x 7 µm)
- Injection volume: 20 µl
- Mobile phase: Acetonitrile:Phosphoric acid-pH 2 (10:90 v/v)
- Flow rate: 2 ml/min

Inject separately 20 µl each of working mixed standard solution and sample solution. Calculate the amount of each monomer in the sample from the peak areas obtained in the chromatograms of working mixed standard solution (rR) and sample solution (rS); amount of standard (R, mg), weight of sample (W, g) and dilution factor (40).

$$\text{Conc. monomer } [\mu\text{g/g}] = \frac{rS \times R \times 40}{rR \times W}$$

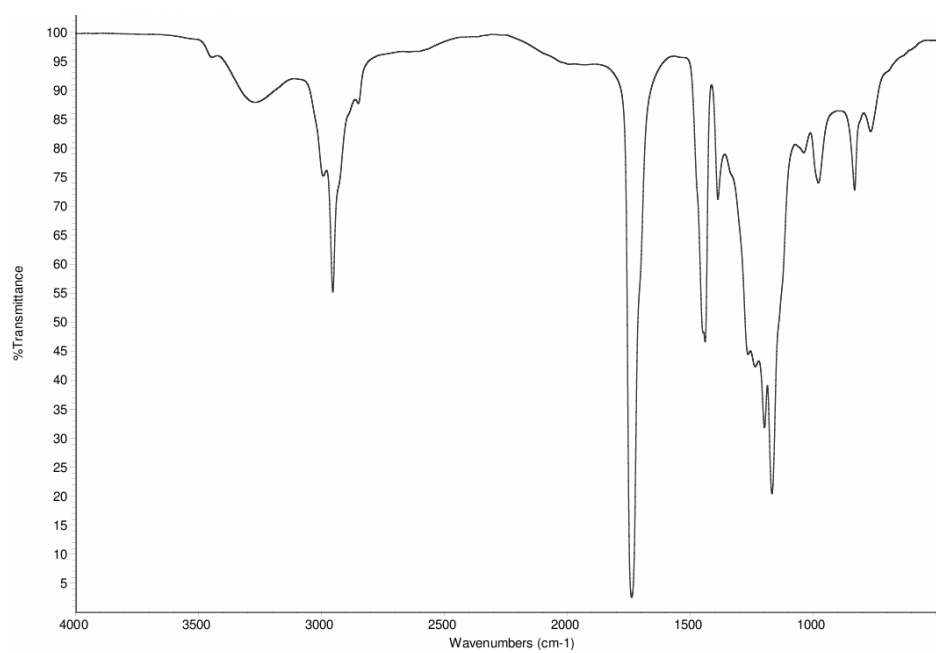
Total monomers in the sample (µg/g) = Sum of monomers in the sample and correct the results for recovery.

METHOD OF ASSAY

Accurately weigh about 5 g sample and dissolve completely in 90 ml isopropyl alcohol and 10 ml water. Titrate with 0.5 N sodium hydroxide standard solution to a potentiometric endpoint. Perform a blank titration under the same conditions. One ml 0.5 N NaOH corresponds to 43.045 mg methacrylic acid units.

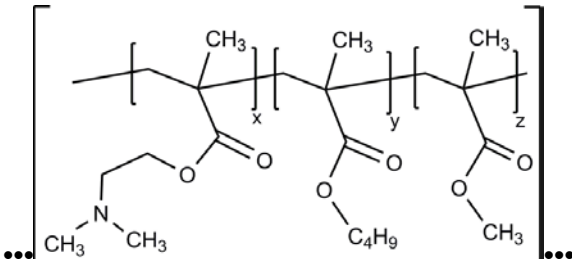
$$\text{Methacrylic acid units (\%w/w, on the dried basis)} = \frac{\text{ml of 0.5 N NaOH} \times 43.045}{\text{sample weight (g)} \times \% \text{ dry substance in sample}}$$

Appendix: Infrared spectrum of anionic methacrylate copolymer



BASIC METHACRYLATE COPOLYMER

New specifications prepared at the 86th JECFA (2018) and published in FAO JECFA Monographs 22 (2018). An ADI of “not specified” was established at 86th JECFA (2018).

SYNONYMS	E 1205; INS No. 1205; 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
DEFINITION	Basic Methacrylate Copolymer is a cationic copolymer comprised of the monomers dimethylaminoethyl methacrylate, butyl methacrylate and methyl methacrylate in the molar ratio of 1:2:1. The copolymer is manufactured by a controlled polymerization process using a free radical donor initiation system. After completion of polymerization, the viscous copolymer solution is fed into an extruder to remove solvents and volatile substances, by actively degassing through vacuum and heating. The solid granules of basic methacrylate copolymer formed in the extruder can be milled to a powder. The copolymer may contain residual monomers (dimethylaminoethyl methacrylate, butyl methacrylate, methyl methacrylate.). Basic methacrylate copolymer is used as a coating and glazing agent for food supplements and foods for special medical purposes.
Chemical name	Poly(butyl methacrylate-co-(2-dimethylaminoethyl)methacrylate-co-methyl methacrylate)
C.A.S. number	24938-16-7
Chemical formula	$\text{Poly}[(\text{CH}_2:\text{C}(\text{CH}_3)\text{CO}_2(\text{CH}_2)_2\text{N}(\text{CH}_3)_2)\text{-co-}(\text{CH}_2:\text{C}(\text{CH}_3)\text{CO}_2\text{CH}_3)\text{-co-}(\text{CH}_2:\text{C}(\text{CH}_3)\text{CO}_2(\text{CH}_2)_3\text{CH}_3)]$
Structural formula	 The structural formula illustrates the copolymer chain with three distinct repeat units. Each unit consists of a methacrylate backbone (CH₂-C(CH₃)-) where the ester group is linked to different side chains: a 2-dimethylaminoethyl group (x), a butyl group (y), and a methyl group (z). The units are connected by ester linkages and the entire chain is enclosed in brackets with ellipses at the ends to indicate the polymer nature.
	The above formula is provided for illustrative purposes; specific repeat units cannot be defined.
Formula weight	47,000 (weight-average), 22,000 (number-average)

Assay 20.8 – 25.5 % dimethylaminoethyl (DMAE) groups on the dried basis

See description under TESTS

DESCRIPTION White powder

FUNCTIONAL USES Coating agent, glazing agent

CHARACTERISTICS

IDENTIFICATION

Viscosity 3 - 6 mPa·s

Determine viscosity using Brookfield viscometer at 20° and 30 rpm using UL adapter.

Refractive index
(Vol. 4) n_D^{20} : 1.380 - 1.385

Solubility (Vol. 4) Freely soluble in methanol, ethanol, and 1 N aqueous hydrochloric acid

Infrared absorption The infrared absorption spectrum of a dry film of sample corresponds to the infrared spectrum in the Appendix.

Apply one drop of sample to a glass plate, cover with a water-resistant crystal disc (AgCl, KRS 5), press lightly, remove the crystal disc and dry for about 15 minutes at 60°.

PURITY

Loss on drying (Vol. 4) Not more than 2.0% (110°, 3 h)

Sulfated ash (Vol. 4) Not more than 0.1%

Test 5 g of the sample (Method I)

Particle size < 50 µm: at least 95 %
< 20 µm: at least 50 %
< 3 µm: not more than 10 %

See description under TESTS

Residual solvents
(Vol. 4) Methanol: Not more than 50 mg/kg;
Butanol: not more than 50 mg/kg and

Propan-2-ol: not more than 100 mg/kg.

(See General Methods, Organic Components, Residual Solvents, Method 1)

Residual monomers Dimethylaminoethyl methacrylate: Not more than 500 mg/kg

Butyl methacrylate: Not more than 100 mg/kg

Methyl methacrylate: Not more than 50 mg/kg

See description under TESTS

Lead (Vol. 4) Not more than 1.0 mg/kg

Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under "General Methods, Metallic Impurities").

Microbiological criteria Total plate count: Not more than 1,000 cfu/g
(Vol. 4)

Yeast and moulds: Not more than 100 cfu/g

Coliforms: Negative in 10 g

TESTS

PURITY TESTS

Particle size Determine using the light diffraction measurement method according to Ph. Eur. 2.9.31

(European Pharmacopeia; Particle Size Analysis by Laser Light Diffraction. 8 01/2010:0333)

Residual monomers **Method for the determination of methyl methacrylate and butyl methacrylate:**

Standards and Reagents:

Phosphate buffer (0.0625 M, pH 2.0):

Prepare an aqueous solution containing 8.9 g of anhydrous dibasic sodium phosphate and 8.5 g of monobasic potassium phosphate in 1 L deionized water. Adjust with phosphoric acid to pH 2.0.

Mobile phase:

Prepare a mixture of methanol and pH 2.0 phosphate buffer (55:45).

Diluent:

Acetonitrile:Buffer (40:60)

Preparation of standard solution

Stock mixed standard solution:

Accurately weigh 20 mg of butyl methacrylate and 10 mg of methyl methacrylate, dissolve in 3 ml of n-butanol and dilute to volume to 10 ml with diluent in a volumetric flask.

Intermediate mixed standard solution:

Pipette 1.0 ml of stock mixed standard solution into a 10 ml volumetric flask and dilute to 10 ml with diluent.

Working mixed standard solution:

Pipette 1.0 ml of intermediate standard solution into a 25 ml volumetric flask and dilute to volume with diluent. This solution contains about 8 µg/ml of butylmethacrylate and 4 µg/ml of methyl methacrylate.

Preparation of Sample solution

Accurately weigh about 1.0 g of sample, dissolve in diluent and make up to 50 ml with diluent and mix.

Procedure

Chromatographic system:

The liquid chromatograph is equipped with a UV/diode array detector capable of working at 205 nm and a column (4.6 mm × 12 cm, packing material: octadecylsilane chemically bonded to porous silica or ceramic microparticles, 1.5-10 µm). Flow rate: 2 ml/min.

Chromatograph the working standard solution, and record the peak responses. The resolution, R, between butyl methacrylate and methyl methacrylate is not less than 10; and the relative standard deviation for replicate injections is not more than 3.0%.

Separately inject 50 µl each of the working standard solution and sample solution and record the peak areas of the monomers.

Calculate the quantity of each monomer in the sample using the formula:

$$\text{Monomer concentration } (\mu\text{g/g}) = (rU/rS) \times (CS/CU) \times F$$

rU = Peak area for the monomer in the sample chromatogram

rS = Peak area for the monomer in the working standard chromatogram

CS = Concentration of monomer in the working standard solution (µg/ml)

CU = Concentration of polymer in the sample solution (mg/ml)

F = Conversion factor (10³ mg/g)

Method for the determination of 2-Dimethylaminoethyl methacrylate

Standards and Reagents:

Monobasic potassium phosphate buffer solution (0.025M):

Prepare an aqueous solution containing 3.4 g of monobasic potassium phosphate per litre.

Mobile phase:

Tetrahydrofuran:monobasic potassium phosphate buffer solution (75:25).

Preparation of standard solution

Stock standard solution (200 µg/ml):

Accurately weigh about 20 mg of (2-dimethylaminoethyl) methacrylate, dissolve in tetrahydrofuran, make up to volume in a 10 ml volumetric flask with tetrahydrofuran and mix.

Working standard solution (8 µg/ml):

Dilute 2.0 ml of the stock standard solution to 50 ml in a volumetric flask with tetrahydrofuran and mix.

Preparation of Sample solution

Accurately weigh about 1.0 g of sample, dissolve in tetrahydrofuran, dilute to 50 ml with tetrahydrofuran in a volumetric flask and mix.

Procedure

Chromatographic system:

The liquid chromatograph is equipped with a UV/diode array detector capable of working at 215 nm and a column (4.6 mm × 12 cm, packing material: an essentially monomolecular layer of aminopropylsilane chemically bonded to totally porous silica gel support, 1.5-10 µm) .
Flow rate: 2 ml/min.

Chromatograph the working standard solution, and record the peak area. The relative standard deviation for replicate injections is not more than 2.0%.

Separately inject 50 µl of the working standard solution and the sample solution and record the peak areas.

Calculate the quantity of each monomer in the sample using the formula”

$$\text{2-Dimethylaminoethyl methacrylate, (}\mu\text{g/g)} = (rU/rS) \times (CS/CU) \times F$$

rU = Peak area for the monomer in the sample chromatogram

rS = Peak area for the monomer in the standard chromatogram

CS = Concentration of monomer in the working standard solution ($\mu\text{g/ml}$)

CU = Concentration of polymer in the sample solution (mg/ml)

F = Conversion factor (10^3 mg/g)

METHOD OF ASSAY Determine the percentage of Dimethylaminoethyl (DMAE) groups using a potentiometric titration.

Dissolve 200 mg of dried sample in 4 ml water and 96 ml of glacial acetic acid. Titrate with 0.1 N standard perchloric acid solution to a potentiometric end point. Perform a blank determination.

$$\begin{aligned} \text{DMAE groups (\%w/w, on the dried basis)} \\ = \frac{(VS - VB) \times N \times F}{W} \times 100 \end{aligned}$$

VS = titrant volume consumed by the sample (ml)

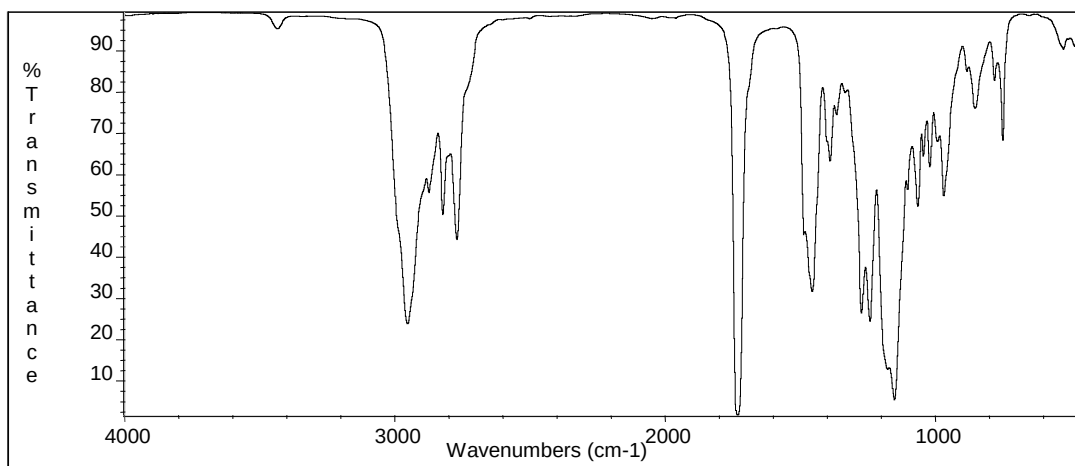
VB = titrant volume consumed by the blank (ml)

N = actual normality of the titrant (mEq/ml)

F = equivalency factor, 72.1 mg/mEq

W = dried sample weight (mg)

Appendix: Infrared spectrum of basic methacrylate copolymer



CASSIA GUM

Prepared at the 86th JECFA (2018) and published in FAO JECFA Monographs 22 (2018), superseding tentative specifications prepared at the 82nd JECFA (2016) and published in FAO JECFA Monographs 19 (2016). An ADI “not specified” was established at the 71st JECFA (2009)

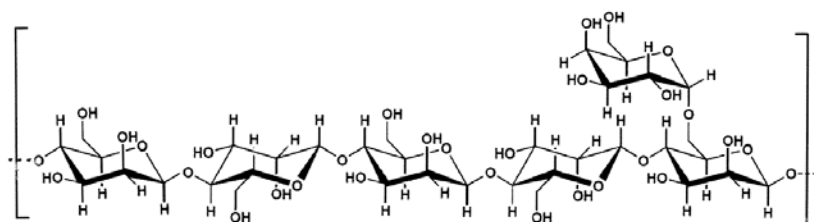
SYNONYMS

INS 427

DEFINITION

Cassia gum is obtained from the ground purified endosperm of the seeds of *Cassia tora* and *Cassia obtusifolia* (Fam. *Leguminosae*) containing less than 0.05% of *Cassia occidentalis*. It consists mainly of high molecular weight (approximately 200,000-300,000) polysaccharides composed of galactomannans with a mannose:galactose ratio of about 5:1. The seeds are dehusked and degermed by thermal and mechanical treatment followed by milling and screening of the endosperm. The ground endosperm is purified by extraction with isopropanol.

Structural formula



The structure above is provided for illustrative purposes. A specific repeat unit for Cassia gum cannot be defined.

Assay

Not less than 75% of galactomannans

DESCRIPTION

Pale yellow to off-white, odourless free-flowing powder. Forms colloidal solutions in cold water.

FUNCTIONAL USES

Thickener, emulsifier, foam stabilizer, moisture retention agent, and texturizing agent.

CHARACTERISTICS

IDENTIFICATION

Solubility

Insoluble in ethanol

Gel formation with borate

Add sodium borate TS to an aqueous dispersion of the sample to raise the pH above 9; a gel is formed.

<u>Gel formation with xanthan gum</u>	Passes test See description under TESTS
<u>Gum constituents</u> (Vol. 4)	Proceed as directed under 'Gum Constituents Identification' using 100 mg of sample (instead of 200 mg) and 1-10 µl of the hydrolysate (instead of 1-5 µl). Use galactose and mannose as reference standards. These constituents should be present.
<u>Viscosity</u>	Less than 500 mPa × s See description under TESTS
<u>pH</u> (Vol. 4)	5.5-8.0 (1% solution)
PURITY	
<u>Loss on drying</u> (Vol. 4)	Not more than 12% (105°, 5 h)
<u>Total ash</u> (Vol. 4)	Not more than 1.2%
<u>Acid-insoluble matter</u> (Vol. 4)	Not more than 2.0%
<u>Protein</u> (Vol. 4)	Not more than 7.0% Multiply percent nitrogen by 6.25.
<u>Crude fat</u>	Not more than 1% See description under TESTS
<u>Starch</u>	To a 1 in 10 dispersion of the sample add a few drops of iodine TS; no blue colour is produced.
<u>Anthraquinones</u>	Not more than 0.5 mg/kg See description under TESTS
<u>Residual solvents</u> (Vol. 4)	Isopropanol: Not more than 1.0% See description under TESTS
<u>Lead</u> (Vol. 4)	Not more than 1 mg/kg Determine using a method appropriate to the specified level. The selection of sample size and method of sample

preparation may be based on the principles of the method described in Volume 4 (under "General Methods, Metallic Impurities").

Microbiological criteria
(Vol. 4)

Total plate count: Not more than 5,000 cfu/g

Yeast and mould: Not more than 100 cfu/g

E. coli: Negative in 1 g

Salmonella: Negative in 25 g

TESTS

IDENTIFICATION TESTS

Gel formation with xanthan gum

Weigh 1.5 g of sample, 1.5 g of xanthan gum and blend them. Add this blend (with rapid stirring) to 300 ml water at 80° in a 400 ml beaker. Stir until the mixture is dissolved and continue stirring for an extra 30 min after dissolution (maintain the temperature above 60° during the stirring period). Discontinue stirring and allow the mixture to cool to room temperature for at least 2 h.

A firm, viscoelastic gel forms after the temperature drops below 40°, but no such gel forms in a 1% control solution of cassia gum or xanthan gum alone prepared in a similar manner.

Viscosity

Add 495 ml of deionized water into a 1L beaker, insert a magnetic stir bar and place the beaker on a magnetic stirrer equipped with a heater. Adjust the stirrer speed to about 750 rpm. Weigh 5 g of sample and quickly add to the beaker. Switch on the heater and heat the beaker to reach 90° and keep it at 90° for 15 min. Cool the solution to room temperature (25° ±1.5°) in a water bath. Measure the viscosity at 25°, after 2 h, using a RVT Brookfield Spindle 1 and 20 rpm speed. Repeat the procedure with a sample of 5 g of carob (locust) bean gum. The viscosity of the cassia gum (150 – 500 mPa × s) must be less than 50% that of carob bean gum (2000 - 3500 mPa × s)

PURITY TESTS

Residual solvents

Determine residual solvents using headspace gas chromatography (Vol. 4; Method I) under the following conditions.

Internal standard solution

Add 50.0 ml water to a 50 ml vial and seal. Accurately weigh and inject 15 µl of 3-methyl-2-pentanone through the septum and reweigh the vial to within 0.01 mg.

Standard solution

Add 50.0 ml water to a 50 ml vial and seal weigh accurately. Inject 15 µl isopropanol and reweigh the vial.

Blank solution:

Add 5.0 ml of water and pipette 1.0 ml of the internal standard solution into a headspace vial. Seal the vial and mix the contents using a vortex mixer.

Calibration solution:

Add 4.0 ml of water into the headspace vial. Pipette 1.0 ml each of the internal standard solution and the standard solution. Seal the vial and mix the contents using a vortex mixer.

Preparation of sample:

Pipette 5 ml of water and 1 ml internal standard solution into a headspace vial. Accurately weigh 0.500 ± 0.001 g of sample in a small weighing boat and add the sample carefully to prevent clumping of sample at the bottom of the vial. Seal the vial and mix the contents using a vortex mixer. Do not shake the sample vial.

Follow the procedure described in Vol. 4 for the determination of residual solvents.

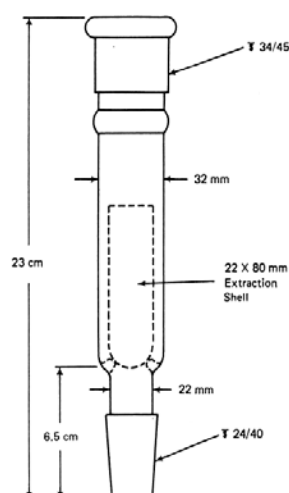
Crude fat

Apparatus

The apparatus consisting of a Butt-type extractor, as shown below, having a standard-taper 34/45 female joint at the upper end, to which is attached a Friedrichs- or Hopkins-type condenser, and a 24/40 male joint at the lower end, to which is attached a 125-ml Erlenmeyer flask.

Procedure

Transfer about 10 g of the sample, previously ground to 20-mesh or finer and accurately weighed, to a cellulose thimble or a 15-cm filter paper (roll the paper tightly around the sample), and place it in a suitable extraction shell). Plug the top of the thimble or the extraction shell with cotton previously extracted with hexane, and place it in the extractor. Attach the extractor to a dry 125-ml Erlenmeyer flask containing about 50 ml of hexane and to a water-cooled condenser, apply heat to the flask to produce 150 to 200 drops of condensed solvent per min, and extract for 16 h. Disconnect the flask, and filter the extract to remove any insoluble residue. Rinse the flask and filter with a few ml of hexane, combine the washings and filtrate in a tared flask, and evaporate on a steam bath until no odour of solvent remains. Dry in a vacuum for 1 h at 100°, cool in a desiccator, and weigh.



Butt-Type Extractor for fat determination.

NOTE: The method for crude fat is referenced from Appendix X: Crude fat in the Food Chemicals Codex, 11th Edition, 2018. Reproduced from USP-NF with permission from The U.S. Pharmacopeial Convention (USP)

Anthraquinones

Principle

Anthraquinones are extracted with chloroform and determined by High Performance Liquid Chromatography (Vol.4) using the conditions below.

NOTE: Anthraquinones are photosensitive. Samples and standards shall be protected from light and all manipulations shall be carried out under the subdued light.

Standards and Reagents:

Emodin, Aloe-emodin, Physcion (1,8-dihydroxy-3-methoxy-6-methyl-anthraquinone), Rhein and Chrysophanic acid (>99%).

Internal standard: Danthrone (1,8-dihydroxy-anthraquinone, >99%)

Methanol, acetonitrile, deionized water, chloroform, trifluoroacetic acid, sulfuric acid and sodium hydrogen carbonate

Individual stock standard and internal standard solutions (100 µg/ml)

Accurately weigh about 10 mg of the standards and internal standard, transfer to 100 ml volumetric flasks with about 5 ml of methanol, sonicate for 15 min and dilute to volume with methanol.

Store these solutions in amber coloured bottles at 4° (the solutions are stable for 2 weeks under these conditions).

Internal spike standard solution (20 µg/ml)

Dilute 2 ml of internal standard stock solution to 10 ml with methanol.

Mixed standard solution (10 µg/ml)

Transfer 1 ml of each of the anthraquinones stock standard solution into a 10 ml volumetric flask and dilute to volume with methanol.

Working standard solutions

To each of five 10 ml volumetric flasks transfer 0, 0.5, 1, 2 and 5 ml respectively of the mixed standard solution, and 1 ml of the internal spike standard solution (20 µg/ml), dilute to volume with methanol and mix.

Sample preparation:

Accurately weigh about 4.0 g of the sample into a 250 ml Erlenmeyer flask. Add 80 µl of internal standard solution (100 µg/ml), and 100 ml 2N H₂SO₄ to the flask. Stopper the flask using a PTFE stopper and heat at 103° for 3.5 hours in an oven. After cooling to room temperature, add 100 ml of chloroform and shake well. Allow phase separation. Evaporate 50 ml of the chloroform layer to dryness in a rotary evaporator at 68°. Dissolve the residue in 2 ml of methanol. Filter the solution through a PTFE membrane syringe filter.

Chromatographic conditions:

Column: Hypersil ODS C18 (250 mm x 4.6 mm ID, 5 µm)

Mobile phase:

(A) 0.1 % trifluoroacetic acid in water

(B) Acetonitrile

Injection volume: 20 µl

Gradient:

Time, min	% (A)	% (B)
0	86	14
10	86	14
15	80	20
25	80	20
55	20	80
60	0	100
66	86	14

Flow rate: 1 ml/min

Detector: Photodiode Array/UV Detector operated at 435 nm.

Procedure

Inject individual standard solutions and internal standard solution (dilute, if required) and record retention times.

Construction of standard curves

Inject 20 µl of each working standard solution.
Construct the standard curves by plotting the ratios of the peak areas of each of the specific anthraquinone / internal standard against the concentrations of each working standard solution (µg/ml).

Inject 20 µl of the Sample solution. Calculate the ratios of the peak areas of each anthraquinone / internal standard, and obtain the amount (A) of each anthraquinone from the respective standard curve.

Concentration of anthraquinone in the sample (µg/g) = $(A \times 4) / W$

Where

A = the amount of each anthraquinone (µg) obtained from the standard curve

W = Mass of sample (g)

4 = Dilution factor for sample

METHOD OF ASSAY

$$\% \text{ Galactomannans} = 100 - (L + A + I + P + F)$$

L % Loss on Drying

A %Total Ash

I %Acid-Insoluble Matter

P %Protein

F %Crude Fat

CITRIC AND FATTY ACID ESTERS OF GLYCEROL (TENTATIVE)

Prepared at the 86th JECFA (2018) and published in FAO JECFA Monographs 22 (2018), superseding specifications prepared at the 82nd JECFA (2016), and published in FAO JECFA Monographs 19 (2016). An ADI 'not limited' was established at the 17th JECFA (1973)

Information required:

- *A validated method for the determination of total citric acid content*
- *Performance characteristics (method validation data) of the citric acid determination method*
- *Data on the total citric acid content, in at least five batches of products currently available in commerce, determined using the above method.*

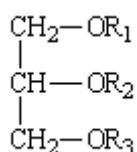
SYNONYMS

Citric acid esters of mono- and di-glycerides, citroglycerides, CITREM; INS No. 472c

DEFINITION

Citric and fatty acid esters of glycerol (CITREM) consists of mixed esters of citric acid and edible fatty acids with glycerol. It may contain free fatty acids, glycerol, citric acid and mono- and diglycerides, in minor quantities. The mono- and di- glycerides may include either one or two edible fatty acids from C12:0 to C18:0, mainly the saturated palmitic (C16:0) and stearic (C18:0) acids. It may also contain minor amounts of other fatty acids such as myristic (C14:0), oleic (C18:1), linoleic (C18:2) and arachidic acid (C20:0). CITREM is obtained by esterification of glycerol with citric acid and edible fatty acids, or by reaction of a mixture of mono- and diglycerides of edible fatty acids, with citric acid. CITREM may be partially or wholly neutralized with sodium hydroxide or potassium hydroxide.

Structural formula



Where at least one of R₁, R₂ or R₃ represents a citric acid moiety, one represents a fatty acid moiety and the remainder may represent citric acid, fatty acid or hydrogen.

DESCRIPTION

White to ivory coloured, oily to waxy material.

FUNCTIONAL USES

Stabilizer, emulsifier, dough conditioner, antioxidant synergist

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water; soluble in oils and fats; insoluble in ethanol

Test for fatty acids
(Vol. 4) Passes test

Test for citric acid Information required

Test for glycerol
(Vol. 4) Passes test

PURITY

Sulfated ash (Vol. 4) Non-neutralized products: not more than 0.5%
Partially or wholly neutralized products: not more than 10%; test 2 g of the sample (Method I)

Free glycerol (Vol. 4) Not more than 4%

Total glycerol 8-33%
See description under TESTS

Total citric acid 13-50%
(Information required)

Total fatty acid 37-81%
See description under TESTS

Lead (Vol. 4) Not more than 2 mg/kg.
(Not more than 0.1 mg/kg for use in infant formula and formula for special medical purposes intended for infants)

Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under "General Methods, Metallic Impurities").

TESTS

PURITY TESTS

Total glycerol

CITREM is hydrolyzed, glycerol in the aqueous phase is oxidized using known excess of sodium periodate in a strongly acid medium and the unreacted periodate is back titrated using standard sodium thiosulfate solution.

Procedure:

Accurately weigh about 2 g of the sample into a saponification flask, add 50 ml of 0.5 M ethanolic potassium hydroxide, and reflux for 30 min.

To a 1-L volumetric flask add 99 ml $\pm$ 0.2 ml of chloroform using a burette and add 25 ml of glacial acetic acid using a graduated cylinder. Quantitatively transfer the content of the saponification flask to the volumetric flask, using three 25 ml portions of water. Add about 500 ml of water further, and shake vigorously for about 1 min. Dilute to volume with water, stopper, mix thoroughly and set aside for separation of layers.

Pipet 50 ml of acetic periodic acid TS into a series of 400 ml beakers. Prepare two blanks by adding 50 ml of water to each. Pipet 50 ml of the aqueous layer into one of the 400 ml beakers containing 50 ml of acetic periodic acid TS; shake gently to mix; cover with watch glass, and allow to stand 30 min but not longer than 1.5 h. Add 20 ml of 15% potassium iodide solution, shake gently to mix, and allow to stand at least 1 min. but not more than 5 min. Do not allow to stand in bright or direct sunlight. Add 200 ml of water and titrate with 0.1 N sodium thiosulfate. Use a variable speed electric stirrer to keep the solution thoroughly mixed. Continue the titration to the disappearance of the brown iodine colour from the aqueous layer. Add 2 ml of starch TS and continue the titration to the disappearance of iodine from the tiny chloroform layer separated during titration and the disappearance of the blue iodine-starch complex colour from the aqueous layer. Read the burette to the nearest 0.01 ml. Treat the blanks in the same way as the sample.

Calculation

$$\% \text{ total glycerol} = [(B - S) \times N \times 2.302 \times 900] / (W \times 50)$$

where

- B volume of 0.1 N sodium thiosulfate used for the blank, ml
- S volume of 0.1 N sodium thiosulfate used for the sample, ml
- N exact normality of 0.1 N sodium thiosulfate
- W mass of sample, g

Total citric acid

Information required

Total fatty acid

Principle: This method measures total fatty acids by extracting with diethyl ether.

Procedure

Weigh accurately 5 g of the sample into a 250-ml round-bottomed flask, add 50 ml of potassium hydroxide, ethanolic, TS, and reflux for 1 h on a boiling water bath.

Quantitatively transfer the contents of the saponification flask to a 1,000 ml separating funnel, using three 25 ml portions of water, and add 5 drops of methyl orange indicator solution.

Cautiously add 50% hydrochloric acid until the colour of solution changes to orange red.t. Add 1 ml of excess acid. Shake well to mix the contents and separate the fatty acids.

Cool to room temperature and extract the separated fatty acids with three 100 ml portions of diethyl ether. Combine the extracts, and wash with 50 ml portions of 10% sodium chloride solution until the washed sodium chloride solution becomes neutral.

Dry the ether solution with anhydrous sodium sulfate. Then evaporate off ether on a steam bath, leave additional 10 min on the steam bath, and weigh the residue. This is the weight of the total fatty acids.

Calculation:

$$\text{Total Fatty acids \%} = \frac{\text{mass of fatty acids g} \times 100}{\text{mass of sample g}}$$

ERYTHROSINE

Prepared at the 86th JECFA and published in FAO JECFA Monograph 22 (2018) superseding specifications prepared at the 41st JECFA (1993), published in FNP 52 Add 2 (1993). Metals and arsenic specifications revised at the 59th JECFA (2002). An ADI of 0-0.1 mg/kg bw was established at the 37th JECFA (1991) and confirmed at the 86th JECFA (2018).

SYNONYMS

INS No. 127, CI Food Red 14, CI (1975) No. 45430, Food Red No. 3, FD&C Red No. 3

DEFINITION

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.

Erythrosine may be converted to the corresponding aluminium lake in which case only the requirements in the *General Specifications for Aluminium Lakes of Colouring Matters* apply.

Chemical names

Disodium 2-(2,4,5,7-tetraiodo-6-oxido-3-oxoxanthen-9-yl)benzoate monohydrate;

Disodium;2',4',5',7'-tetraiodo-3-oxospiro[2-benzofuran-1,9'-xanthene]-3',6'-diolate;

Disodium 2',4',5',7'-tetraiodofluorescein monohydrate

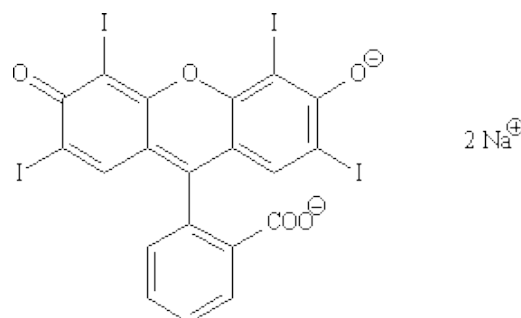
C.A.S. number

16423-68-0

Chemical formula

$C_{20}H_6I_4Na_2O_5 \cdot H_2O$

Structural formula



Formula weight

879.86

Assay

Not less than 87% total colouring matters

DESCRIPTION	Red powder or granules
FUNCTIONAL USES	Colour
CHARACTERISTICS	
IDENTIFICATION	
<u>Solubility</u> (Vol. 4)	Soluble in water, slightly soluble in ethanol
<u>Spectrophotometry</u> (Vol. 4)	Maximum wavelength approximately 527 nm Determine the UV-visible absorption spectrum of the sample dissolved in water.
PURITY	
<u>Loss on drying, chloride and sulfate as sodium salts</u> (Vol. 4)	Not more than 13% Determine chloride as sodium chloride, sulfate as sodium sulfate, and loss on drying (135°, 6 h) as described in Volume 4 (under "Specific Methods, Food Colours").
<u>Inorganic iodides</u>	Not more than 0.1% calculated as sodium iodide See description under TESTS
<u>Water insoluble matter</u> (Vol. 4)	Not more than 0.2%
<u>Zinc</u> (Vol. 4)	Not more than 50 mg/kg Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4 (under "General Methods, Metallic Impurities").
<u>Lead</u> (Vol. 4)	Not more than 2 mg/kg Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4 (under "General Methods, Metallic Impurities").
<u>Subsidiary colouring matters</u>	Not more than 4% (except fluorescein) See description under TESTS

Note: Do not allow the sample and standard solutions to be exposed to direct sunlight.

Fluorescein

Not more than 20 mg/kg

See description under TESTS

Organic compounds
other than colouring
matters

Triiodoresorcinol: Not more than 0.2%

2-(2,4-dihydroxy-3,5-diiodobenzoyl)benzoic acid: Not more than 0.2%

See description under TESTS

Ether extractable
matter (Vol. 4)

From a solution of pH not less than 7, not more than 0.2%

Hydrochloric acid-
insoluble matter in
Erythrosine Lake

Not more than 0.5%

See description under TESTS

TESTS

PURITY TESTS

Inorganic iodides

Weigh 1.0 g of the sample into a 100-ml beaker. Add 75 ml distilled water and a magnetic stirrer. Stir to dissolve. Immerse an iodide specific electrode and a reference electrode in the solution and use a suitable millivoltmeter to read the potential of the system in millivolts.

Add 0.001 M silver nitrate solution from a burette initially in 0.5 ml aliquots, reducing these to 0.1 ml as the end-point approaches as indicated by an increasing change in potential for each addition. After allowing time for the reading to stabilize, record the millivolt readings after each addition. Continue the titration until further additions make little change in the potential.

Plot the millivolt readings against the volume of silver nitrate solution added. The equivalence point is the volume corresponding to the maximum slope of the curve.

The percentage of sodium iodide in the sample = $\text{Titre} \times 0.015\%$

where

Titre ml-equivalent of silver nitrate solution

0.015% $0.001 \text{ mol/l} \times 10^{-3} \text{ l/ml} \times 149.89 \text{ g sodium iodide/mol} \times 1 \text{ mol/equivalent} \times 1/1.0 \text{ g (sample weight)} \times 100.$

Subsidiary colouring matters

Determine subsidiary colouring matters content by reversed-phase HPLC (Vol. 4) using the following conditions:

- Column: C8 (250 mm x 4.6 mm i.d., 5 µm particle size)
- Eluent A: 0.1 M ammonium acetate in water
- Eluent B: methanol
- Injection volume: 20 µl
- Column temperature: ambient
- Detector: UV-visible/diode array at 514 nm
- Flow rate: 1.0 ml/min

Gradient:

Elution time (min)	Eluent A (%)	Eluent B (%)
0	55	45
20	34	66
21.1	0	100
25.5	0	100
26.0	55	45
40.0	55	45

Reagents: HPLC grade

Standards:

- 2',4',5'-Triiodofluorescein (C.A.S. 56254-06-9) – synthesized material (see Appendix)
- 2',4',7'-Triiodofluorescein (C.A.S. 83498-90-2) – synthesized material (see Appendix)
- 4',5'-Diiodofluorescein, disodium salt (C.A.S. 33239-19-9) – Alfa Aesar, Cat. No. A15626 or equivalent
- 2'-Monoiodofluorescein, disodium salt (C.A.S. 52010-85-2) – synthesized material (see Appendix)
- 4'-Monoiodofluorescein, disodium salt (C.A.S. 52010-86-3) – synthesized material (see Appendix)
- Erythrosine (C.A.S. 16423-68-0) – TCI, >95.0% disodium 2',4',5',7'-tetraiodofluorescein, Cat. No. F0139 or equivalent (use if subsidiary colouring matter standards are not available)

Prepare standard solutions as required.

Sample preparation:

Weigh accurately 200 ± 2 mg sample and dissolve in 100 ml of water. Dilute the solution, if required, to separate subsidiary colours from the primary colour component in order to improve their resolution.

Calculations:

Construct the relevant standard curves. Integrate all peaks of the chromatogram obtained at 514 nm. If Erythrosine is used as a standard, calculate the ratio of the sum of all peaks not corresponding to Erythrosine to the sum of all peaks.

Fluorescein

Determine fluorescein by the test for subsidiary colouring matters content except use the following conditions:

- Injection volume: 50 μ l
- Detector: UV-visible/diode array at 492 nm

Standard: Fluorescein, disodium salt (C.A.S. 518-47-8) – TCI, Cat. No. F0096 or equivalent

Sample preparation:

Weigh accurately 2.00 ± 0.05 g sample and dissolve in 10 ml of water.

Organic compounds other than colouring matters

Determine organic compounds other than colouring matters by reversed-phase HPLC (Vol. 4) using the following conditions:

- Column: C18 (150 mm x 2.1 mm i.d., 5 μ m particle size)
- Eluent A: 0.05 M sodium dihydrogen phosphate in 95/5 water/methanol, pH 4.0
- Eluent B: methanol
- Injection volume: 5 μ l
- Column temperature: 27°
- Detector: UV-visible/diode array at 223 nm
- Flow rate: 0.5 ml/min

Gradient:

Elution time (min)	Eluent A (%)	Eluent B (%)
0	95	5
3	95	5
5	80	20
13	35	65

15	0	100
25	0	100
27	95	5
37	95	5

Reagents: HPLC grade

Standards:

- 2,4,6-Triiodoresorcinol (C.A.S. 19403-92-0) – Alfa Chemistry, Cat. No. ACM19403920 or equivalent
- 2-(2,4-Dihydroxy-3,5-diiodobenzoyl)benzoic acid (C.A.S. 3480-21-5) – Wako, Cat. No. 043-32981 or equivalent

Prepare standard solutions as required. Dissolve the standards in methanol. Use an amber glass volumetric flask for 2,4,6-triiodoresorcinol and prepare the standard and calibration solutions immediately before use.

Sample preparation:

Weigh accurately 100±5 mg sample and dissolve in 10 ml of methanol.

Calculations:

Construct the relevant standard curves. Integrate all peaks of the chromatogram obtained at 223 nm.

Hydrochloric acid-insoluble matter in Erythrosine Lake

Reagents

- Concentrated hydrochloric acid
- Hydrochloric acid, 0.5% v/v
- Dilute ammonium hydroxide solution (dilute 10 ml of 14.5 M ammonium hydroxide to 100 ml with water).

Procedure

Accurately weigh approximately 5 g of the lake into a 500-ml beaker. Add 250 ml water and 60 ml concentrated hydrochloric acid. Boil to dissolve the alumina while the Erythrosine converts to its "free acid" form, which is insoluble in acid. Filter through a tared No. 4 sintered glass crucible. Wash the crucible with a small amount of hot 0.5% hydrochloric acid and then with some hot distilled water. Remove the acid filtrate from the filter flask, replace the crucible, and wash with hot dilute ammonium hydroxide solution until the washings are colourless. Dry the crucible to constant weight at 135°. Express the residue as a percentage of the weight taken.

METHOD OF ASSAY

Determine total colouring matters content by spectrophotometry using Procedure 1 in Volume 4 (under “Specific Methods, Food Colours”) and an appropriate solvent.

Using water as the solvent:

absorptivity (a) = 110 l/(g × cm)

wavelength of maximum absorbance = 527 nm.

GLYCEROL ESTER OF WOOD ROSIN

Prepared at the 86th JECFA and published in FAO JECFA Monographs 22 (2018), superseding specifications prepared at the 77th JECFA (2013) and published in FAO JECFA Monographs 14 (2013). An ADI of 0-25 mg/kg bw for glycerol ester of wood rosin was established at the 77th JECFA (2013).

SYNONYMS INS No. 445(iii)

DEFINITION Glycerol ester of wood rosin (GEWR) is a complex mixture of glycerol di- and tri- esters of resin acids from wood rosin, with a residual fraction of glycerol monoesters. In addition, neutrals (non-acidic saponifiable and unsaponifiable substances) and residual free resin acids are present. Wood rosin is obtained by the solvent extraction of aged pine stumps, followed by a liquid-liquid solvent refining process. Refined wood rosin is composed of approximately 90% resin acids and approximately 10% neutrals. The resin acid fraction is a complex mixture of isomeric diterpenoid monocarboxylic acids having the typical empirical formula $C_{20}H_{30}O_2$, of which the main components are dehydroabietic and abietic acids. GEWR is produced by esterifying the resin acids with food grade glycerol. The product is then purified by steam stripping or by direct countercurrent steam distillation.

These specifications do not cover substances derived from gum rosin, an exudate of living pine trees, and substances derived from tall oil rosin, a by-product of kraft (paper) pulp processing.

C.A.S. number 8050-30-4

DESCRIPTION Hard, yellow to pale amber-coloured solid

FUNCTIONAL USES Emulsifier, density adjustment agent (flavouring oils in beverages), stabilizer, plasticizer (in chewing gum bases).

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water, soluble in acetone

Infrared absorption (Vol. 4) The infrared spectrum of a thin film of the sample (potassium bromide disc) corresponds with the typical infrared spectrum below

Sulfur test

Negative

Weigh 40-50 mg of sample into a test tube and add 1- 2 drops of a 20% (w/v) solution of sodium formate. Place a strip of lead acetate test paper over the mouth of the test tube. Heat the tube until fumes are formed that contact the test paper. Continue heating for 2-5 min. The formation of a black spot of lead sulfide indicates the presence of sulfur-containing compounds. (Detection Limit: 50 mg/kg sulfur)

Gas chromatography of resin acids and glycerol

Passes test

See description under TESTS

PURITYSpecific gravity
(Vol. 4)

d (20, 25): Not less than 0.935 (50% solution in d-limonene)

Ring and ball softening point (Vol. 4)

Not less than 82° (see “Specific Methods, Glycerol Esters of Rosins”)

Acid value (Vol. 4)

Between 3 and 9 (see “Specific Methods, Fats, Oils, and Hydrocarbons”)

Lead (Vol. 4)

Not more than 1 mg/kg

Determine using method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4 (under “General Methods, Metallic Impurities”).

TESTSIDENTIFICATION
TESTSGas chromatography of resin acids

The ester groups in the glycerol esters of wood rosin are reduced with a metal hydride to form a mixture of corresponding resin alcohols and glycerol which are analyzed by gas chromatography (Vol. 4). The characteristic chromatogram shows predominant peaks for abietic and dehydroabietic alcohols.

Apparatus

- Gas Chromatograph equipped with a flame ionization detector.
- Centrifuge: table top, capable of achieving 3200 rpm

Standards and reagents

- Internal Standard (1,4-Butanediol: >99%
- Toluene
- Sodium Vitride Reagent [(Sodium bis(2-methoxyethoxy) aluminium dihydride], 70% in toluene:(~ 3.5 mol/l)

Sodium Vitride solution:

Pipet 10.0 ml of sodium vitride reagent into a 100 ml volumetric flask dilute to volume with toluene and mix thoroughly.

Hydrolysis solution:

Slowly add 50 ml of concentrated sulfuric acid, reagent grade, to 200 ml distilled water while stirring in an ice bath. Cool to room temperature.

Procedure

Sample preparation

Weigh 250-300 mg sample into a 25 ml Erlenmeyer flask containing a Teflon coated stirrer bar. Pipet 5.0 ml toluene into the flask and stir until sample is dissolved. Pipet 5.0 ml of sodium vitride solution into the flask, stopper the flask and stir for 30 min. While stirring, pipet 3.0 ml of hydrolysis solution into the flask. Continue stirring for 3 min. Transfer contents of flask to centrifuge tube (15 ml), stopper, and shake vigorously. Vent and centrifuge at 2800-3200 rpm for 5 min. Inject 0.5 µl of the toluene layer into the gas chromatograph operating under the following conditions and record the chromatogram. Compare with the chromatogram shown below to verify the approximate retention order of the resin alcohols.

Chromatographic conditions

- Column: DB-1 methyl silicone (bonded and crosslinked) wide-bore capillary (15 m x 0.53 mm i.d., 1.5 µm).
- Injector: Flash vaporization injector
- Flow rates: Carrier Gas (He): 30 ml/min at 63 psi, Hydrogen: 30 ml/min and
- Air: 240 ml/min
- Temperatures: Column: Isothermal, 190°; Injector: 250°, and Detector: 250°

Gas chromatography of glycerol

Standards and reagents

- Glycerol: >99%
- 1,4-Butanediol (Internal standard): >99%

Internal Standard Solution:

Weigh 0.1 g of 1,4-butanediol into a 100 ml volumetric flask. Dilute to volume with distilled water and mix thoroughly.

Glycerol solution:

Weigh 0.1 g of 1,4-butanediol and 0.1 g glycerol into a 100 ml volumetric flask. Dilute to volume with distilled water and mix thoroughly

Phenolphthalein Solution: 1% in ethanol.**Sodium Hydroxide Solution:**

Dissolve 16 g of reagent grade NaOH in 70-80 ml of distilled water and cool to room temperature. Dilute to 100 ml with distilled water and mix thoroughly. Store in a polyethylene bottle.

Procedure**Sample preparation**

Proceed as in the sample preparation for the analysis of resin acids until the centrifugation step. Using a pipet or syringe, remove the toluene layer and part of the aqueous layer leaving approximately 2 ml of the aqueous layer in the centrifuge tube. Add 1 drop of phenolphthalein solution to the remaining aqueous layer in the centrifuge, and neutralize with the sodium hydroxide solution (aluminium salts will precipitate). Pipet 5 ml of the internal standard solution into the tube, dilute to 15 ml with distilled water, stopper, shake, and then centrifuge at 2800-3200 rpm for 5 min. Inject 1 µl of the clear supernatant liquid into the gas chromatograph operating under the following conditions and record the chromatogram. Inject 1 µl of the glycerol solution and record the chromatogram. Measure the retention times of any observed peaks relative to 1,4-butanediol. Compare retention times to that of glycerol standard.

Chromatographic conditions

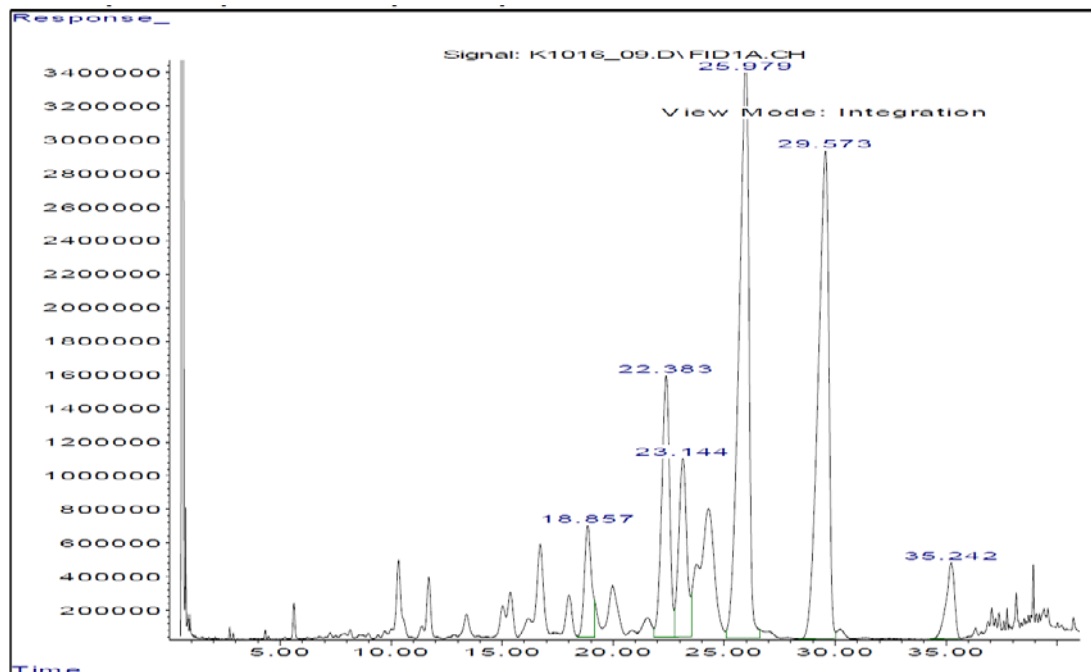
- Column: DB-WAX polyethyleneglycol (bonded and cross-linked), wide bore capillary (15 m x 0.53 mm i.d., 1.0 µm)
- Flow rates: Carrier Gas (He): 30 ml/min at 60 psi, Hydrogen: 30 ml/min and
- Air: 240 ml/min
- Temperatures: Column: Programmed, 120 to 200° at 6°/min; Injector: 250°,
- and Detector: 250°

Gas chromatography of resin acids in GEWR (determined as alcohols)

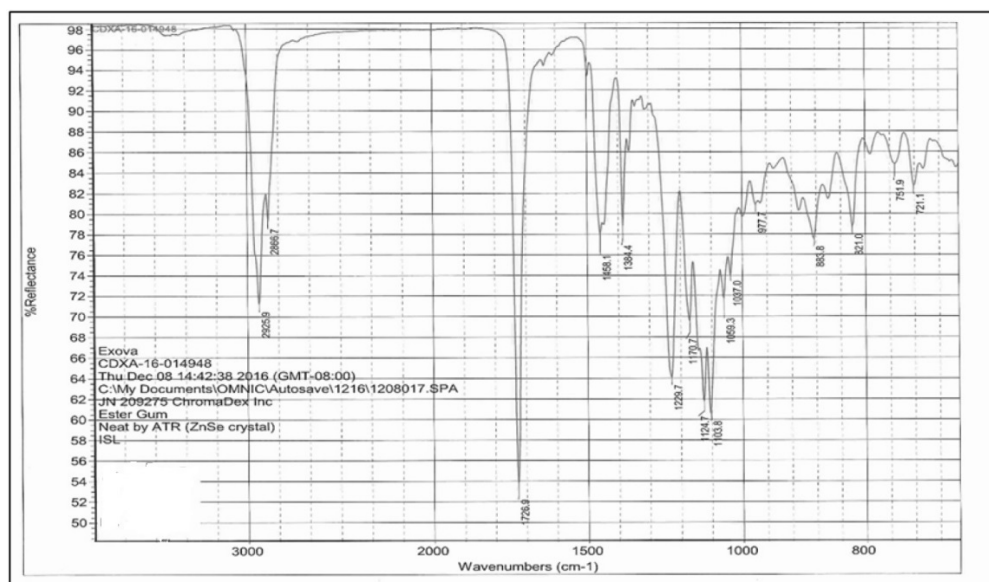
Typical GC-FID chromatogram of a GEWR sample. Retention times correspond to pimaric (18.8 min), isopimaric (22.4 min), palustric (23.1 min), dehydroabietic (25.9 min), abietic (29.6 min), and neoabietic (35.2 min) alcohols. This is a product

derived from a plant-based source which can demonstrate significant variability and relative intensities.

Gas chromatogram



FTIR Spectrum of Glycerol esters of wood rosin



INDIGOTINE

Prepared at the 86th JECFA and published in JECFA Monograph 22 (2018) superseding specifications prepared at the 28th JECFA (1984), published in FNP 31/1 (1984) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). An ADI of 0-5 mg/kg bw was established at the 18th JECFA (1974) and confirmed at the 86th JECFA (2018).

SYNONYMS

INS No. 132, CI Food Blue 1, CI (1975) No. 73015, Indigo Carmine, Food Blue No. 2, FD&C Blue No. 2

DEFINITION

Indigotine consists of a mixture of disodium 3,3'-dioxo-[delta2,2'-biindoline]-5,5'-disulfonate and disodium 3,3'-dioxo-[delta2,2'-biindoline]-5,7'-disulfonate 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.

Indigotine may be converted to the corresponding aluminium lake in which case only the requirements in the *General Specifications for Aluminium Lakes of Colouring Matters* apply.

Chemical names

Disodium 3,3'-dioxo-[delta2,2'-biindoline]-5,5'-disulfonate

Disodium (2E)-3-oxo-2-(3-oxo-5-sulfonato-2,3-dihydro-1H-indol-2-ylidene)-2,3-dihydro-1H-indole-5-sulfonate

Disodium;(2E)-3-oxo-2-(3-oxo-5-sulfonato-1H-indol-2-ylidene)-1H-indole-5-sulfonate

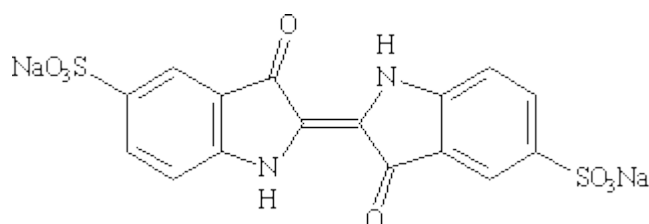
C.A.S. number

860-22-0 (5,5' isomer)

Chemical formula

C₁₆H₈N₂Na₂O₈S₂

Structural formula



Formula weight

466.36

Assay	Not less than 85% total colouring matters
	Not more than 18% of disodium 3,3'-dioxo-[delta2,2'-biindoline]-5,7'-disulfonate
DESCRIPTION	Blue powder or granules
FUNCTIONAL USES	Colour
CHARACTERISTICS	
IDENTIFICATION	
<u>Solubility</u> (Vol. 4)	Soluble in water, sparingly soluble in ethanol
<u>Spectrophotometry</u> (Vol. 4)	Maximum wavelength approximately 610 nm
	Determine the UV-visible absorption spectrum of the sample dissolved in water.
PURITY	
<u>Loss on drying, chloride and sulfate as sodium salts</u> (Vol. 4)	Not more than 15%
	Determine chloride as sodium chloride, sulfate as sodium sulfate, and loss on drying (135°, 6 h) as described in Volume 4 (under "Specific Methods, Food Colours").
<u>Water insoluble matter</u> (Vol. 4)	Not more than 0.2%
<u>Lead</u> (Vol. 4)	Not more than 2 mg/kg
	Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4 (under "General Methods, Metallic Impurities").
<u>Subsidiary colouring matters</u>	Not more than 18% disodium 3,3'-dioxo-[delta2,2'-biindoline]-5,7'-disulfonate (isomeric subsidiary colouring matter)
	Not more than 1% other subsidiary colouring matters
	See description under TESTS
<u>Organic compounds other</u>	Not more than 0.5% of sum of isatin-5-sulfonic acid, 5-

than colouring matters

sulfoanthranilic acid, and anthranilic acid

See description under TESTS

Un sulfonated primary aromatic amines

(Vol. 4)

Not more than 0.01% calculated as aniline

Ether extractable matter

(Vol. 4)

Not more than 0.2%

Weigh accurately about 2 g sample instead of the 5 g stated in the general methods

TESTS

PURITY TESTS

Subsidiary colouring matters

Determine subsidiary colouring matters content by reversed-phase HPLC (Vol. 4) using the following conditions:

- Column: C18 (250 mm x 4 mm i.d., 5 µm particle size)
- Eluent A: 0.2 M ammonium acetate in water
- Eluent B: acetonitrile
- Injection volume: 20 µl
- Column temperature: ambient
- Detector: UV-visible/diode array at 610 nm
- Flow rate: 1.0 ml/min

Gradient:

Elution time (min)	Eluent A (%)	Eluent B (%)
0	100	0
20.0	40	60
30.0	40	60
32.0	100	0
40.0	100	0

Reagents: HPLC grade

Standards:

- Indigotine disodium salt (5,7' isomer) (isomeric subsidiary colour) (C.A.S. 27414-68-2) – Angene Chemical, Cat. No. AGN-PC-0R372R or equivalent
- Sodium indigo sulfonate monosodium salt (monosulfonated subsidiary colour) (C.A.S. 27414-69-3) – Atomax Chemicals Co., Ltd., Cat No. AM27414693 or equivalent

- Trisodium indigo-5,5',7'-trisulfonate
- (trisulfonated subsidiary colour), potassium salt (C.A.S. 67627-18-3) – Sigma-Aldrich, Cat No. 234087 or equivalent
- Indigotine (C.A.S. No. 860-22-0) – TCI, Cat. No. F0148 or equivalent (use if subsidiary colouring matter standards are not available)

Prepare standard solutions as required.

Sample preparation:

Weigh accurately 100 ± 2 mg sample and dissolve in 100 ml of water. Dilute the solution, if required, to separate subsidiary colours from the primary colour component in order to improve their resolution. Analyze immediately after preparation.

Calculations:

Construct the relevant standard curves. Integrate all peaks of the chromatogram obtained at 610 nm. If Indigotine is used as a standard, calculate the ratio of the sum of all peaks not corresponding to Indigotine to the sum of all peaks.

Organic compounds other than colouring matters

Determine organic compounds other than colouring matters content by reversed-phase HPLC (Vol. 4) using the following conditions:

- Column: Luna C18 (250 mm x 4.6 mm i.d., 5 μ m particle size) or equivalent
- Eluent A: 0.1% trifluoroacetic acid in water
- Eluent B: acetonitrile
- Injection volume: 20 μ l
- Column temperature: 25°
- Detector: UV-visible/diode array at 244 nm
- Flow rate: 1.0 ml/min

Gradient:

Elution time (min)	Eluent A (%)	Eluent B (%)
0	100	0
15	80	20
20	0	100
22	0	100
22.1	100	0
32	100	0

Reagents: HPLC grade

Standards:

- Isatin-5-sulfonic acid sodium salt dihydrate (C.A.S. 207399-16-4) – Sigma-Aldrich Cat. No. 58245 or equivalent
- 5-Sulfoanthranilic acid (2-amino-5-sulfobenzoic acid) (C.A.S. 3577-63-7) – TCI Cat No. S0802 or equivalent
- Anthranilic acid (C.A.S. 118-92-3) – Sigma-Aldrich Cat No. A89855 or equivalent

Prepare standard solutions as required.

Sample preparation:

Weigh accurately 100 ± 2 mg sample and dissolve in 100 ml of water. Analyse immediately after preparation.

Calculations:

Construct the relevant standard curves. Integrate the chromatogram peaks obtained at 244 nm.

METHOD OF ASSAY

Determine total colouring matters content by spectrophotometry using Procedure 1 in Volume 4 (under “Specific Methods, Food Colours”) and an appropriate solvent. Analyse immediately after preparation.

Using water as the solvent:

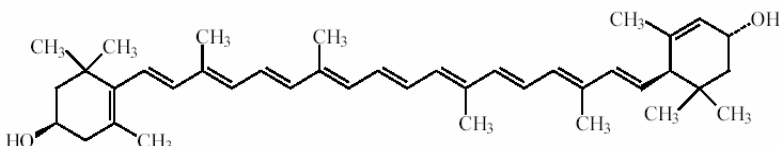
$$\text{absorptivity } (a) = 48.0 \text{ l/(g} \times \text{cm)}$$

wavelength of maximum absorbance = 610 nm.

Determine isomer content by HPLC using the test for subsidiary colouring matters.

LUTEIN FROM TAGETES ERECTA

Prepared at the 86th JECFA (2018) and published in FAO JECFA Monograph 22 (2018), superseding specifications prepared at the 63rd JECFA (2004) and published in FNP52 Add 12 (2004). A group ADI of “not specified” was established for Tagetes extract, Lutein from Tagetes erecta, Lutein esters from Tagetes erecta, Zeaxanthin (synthetic), and meso-zeaxanthin at the 86th JECFA (2018) superseding the group ADI of 0 - 2 mg/kg bw for lutein from T. erecta L. and synthetic zeaxanthin established at the 63rd JECFA (2004).

SYNONYMS	INS No. 161b(i), Vegetable lutein; vegetable luteol; Bo-Xan, luteine
DEFINITION	Lutein from <i>Tagetes erecta</i> is a purified extract of xanthophylls obtained from oleoresin in marigold. The oleoresin is prepared from hexane extracts of <i>Tagetes erecta</i> L. flowers, saponified with potassium hydroxide in either methanol or propylene glycol. The resulting reaction mixture is diluted with water and dried. The crystalline product contains lutein along with minor components that include other carotenoids and waxes.
Chemical names	3R,3'R,6'R- β,ϵ -carotene-3,3'-diol; all- <i>trans</i> -lutein; 4',5'-didehydro-5',6'-dihydro-beta,beta-carotene-3,3'-diol
C.A.S. number	127-40-2
Chemical formula	C ₄₀ H ₅₆ O ₂
Structural formula	
Formula weight	568.88
Assay	Not less than 80% total carotenoids, not less than 70% lutein
DESCRIPTION	A free-flowing, orange-red powder
FUNCTIONAL USES	Colour, nutrient supplement

CHARACTERISTICS**IDENTIFICATION**

<u>Solubility</u> (Vol. 4)	Insoluble in water, soluble in hexane
<u>Spectrophotometry</u> (Vol. 4)	A 2 mg/l solution in acetone shows maximum absorbance at approximately 446 nm.
<u>Test for carotenoids</u> (Vol. 4)	The colour of 2 ml of a 2 – 4 mg/l solution of the sample in acetone immediately disappears after successive addition of about 0.5 ml of 5% sodium nitrite and about 0.5 ml of 0.5 M sulfuric acid.

PURITY

<u>Moisture</u> (Vol. 4)	Not more than 1.0%
<u>Ash</u> (Vol. 4)	Not more than 1.0%
<u>Zeaxanthin</u>	Not more than 9.0%
	See description under METHOD OF ASSAY
<u>Lead</u> (Vol. 4)	Not more than 3 mg/kg.
	Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Vol. 4 (under “General Methods, Metallic Impurities”).
<u>Hexane</u> (Vol. 4)	Not more than 50 mg/kg
<u>Methanol</u> (Vol. 4)	Not more than 10 mg/kg
<u>Propylene glycol</u>	Not more than 1000 mg/kg
	See description under TESTS
<u>Waxes</u>	Not more than 14.0%
	See description under TESTS

TESTS

PURITY TESTS

Propylene glycol

Determine by gas chromatography (Vol. 4) under the following conditions.

Internal standard solution

Prepare a 500 µg/ml solution of ethylene glycol in tetrahydrofuran.

Standard solutions

Prepare a range of standard solutions containing 1, 5, 10, 25 and 50 µg/ml of propylene glycol and 5 µg/ml of ethylene glycol in tetrahydrofuran.

Chromatography conditions

- Column: Polydimethylsiloxane (30 m x 0.32 mm i.d. with 0.25 µm film)
- Carrier gas: Helium
- Flow rate: 1.5 ml/min (Constant flow)
- Detector: FID
- Temperatures: injection port: 230°
- Column Temperature: Hold for 3 min at 40°, then 40-250° at 20°/min, hold for 5 min at 250°
- Detector Temperature: 270°

The retention times of ethylene glycol and propylene glycol derivatives under the above conditions are approx. 7.6 min and 7.8 min, respectively.

Procedure

Weigh accurately 1 g of the sample into a 10-ml volumetric flask, and add 100 µl of the internal standard solution. Dissolve and make up to volume with tetrahydrofuran. Take 0.5 ml of the sample solution in a centrifugation tube, and add 0.25 ml of 1,1,1,3,3,3-hexamethyldisilazane (HMDS) and 0.1 ml of trimethylchlorosilane (TMCS). After sealing the tube, shake it vigorously, let stand for 30 min at room temperature, then centrifuge. Inject 1.0 µl of this centrifugal supernatant into the chromatograph.

Standard curve

Prepare following the same procedure using 0.5 ml of the standard solutions in place of the sample solution.

Calculate the concentration of propylene glycol in mg/kg (C_{PG}) from:

$$\text{CPG (mg/kg)} = C \times 10 / W$$

where

C is polyethylene glycol concentration determined ($\mu\text{g/ml}$); and
W is weight of sample (g)

Waxes

Determine by gas chromatography (Vol. 4) using the following conditions:

- GC column DB-5 (30 m x 0.25 mm ID with a 0.25 μm film thickness) or equivalent.
- GC injector temperature: 280°
- FID temperature: 350°
- GC temperature program: 50° (2 min) 13°/min to 340° and hold for 8 min
- Carrier gas (Helium) flow rate: 1.0 ml/min
- Injection mode: splitless
- Injection volume: 1.0 μl

Standards:

- Hydrocarbons mixed standard: C25 to C46
- Internal standard: Hexatriancontane (C36)

Standard solutions:

Prepare standard solutions by addition of hydrocarbon standards to methylene chloride to get hydrocarbon concentrations of 2.0, 5.0, 10, 25, 50, mg/l respectively. Add required quantity of hexatriancontane internal standard to get a final concentration 50 mg/l in all standard solutions.

Sample Preparation

Accurately weigh 100 mg of sample into a centrifuge tube and dissolve in exactly 20 ml of methylene chloride. Sonication or vortex mixing may be required to completely dissolve the product. Centrifuge sample at 2500 rpm for 5 min, if the sample appears turbid. Add 1.6 ml of methylene chloride and 20 μl of (5000 mg/l) hexatriancontane solution (to a final concentration of 50 mg/l) into 2 ml volumetric flask. Transfer 40 μl of sample solution and dilute with methylene chloride to the 2 ml. Transfer the solution into a 2 ml autosampler vial.

Analysis

Inject 1.0 μl of each of the standards solutions. Record the peak areas. Construct standard curves using the peak ratios of each hydrocarbon to the internal standard against the concentration of the hydrocarbon. Inject 1.0 μl of the sample solution and determine individual wax in the sample (mg/l) from the respective standard curve.

Add the concentration of individual waxes to get the total wax concentration in the sample solution (mg/l)

Calculation:

$$\begin{aligned}\text{Waxes \% w/w} &= \frac{C \text{ (mg/l)} \times 2 \text{ ml} \times 20 \text{ ml} \times 100}{1000 \text{ (ml/l)} \times W \text{ (mg)} \times 0.04 \text{ ml}} \\ &= (100 \times C)/W\end{aligned}$$

Where:

C is the total concentration of waxes, mg/l in the sample

W is the weight of sample, mg

METHOD OF ASSAY

Determine the total carotenoid content and the content of lutein and zeaxanthin by UV spectrophotometry and HPLC using the following conditions:

Reagents:

- Hexane (HPLC grade)
- Ethyl acetate (HPLC grade)
- Acetone
- Dehydrated ethyl alcohol (absolute alcohol)
- Toluene
- Solvent Mixture: (10:6:7:7 hexane:dehydrated ethyl alcohol:acetone:toluene, v/v/v/v)

System Suitability Solution for HPLC:

150 µg/ml of lutein standard in solvent mixture (use USP Lutein RS available from U. S. Pharmacopeia, or equivalent standard)

Apparatus

UV/Vis spectrophotometer; 1-cm cuvettes
HPLC system with suitable diode array detector, autosampler, column oven, signal processor and degasser.
Analytical column: 3 µm silica, 4.6 mm x 250 mm

Instrument Conditions

- Temperature: ambient
- Mobile Phase: 70:30 (v:v) hexane/ethyl acetate (isocratic elution)
- Flow Rate: 1.5 ml/min
- Injection: 10 µl
- Detection: UV/Vis 446 nm
- Run Time: approximately 40 min

Concentrated Sample Preparation

For the UV/Vis spectrophotometry weigh sample (30 mg) into a glass weighing funnel. Using the solvent mixture, wash crystals into a 100 ml volumetric flask, dilute to the mark with the solvent mixture and stir for 10 min.

Sample Preparation

Pipette 1 ml of concentrated sample preparation into a

100 ml volumetric flask. Dilute up to the mark with dehydrated ethyl alcohol, mix by inversion for 20 seconds. Read samples in a spectrophotometer at 446 nm using dehydrated ethyl alcohol as the blank.

For HPLC, evaporate 1 ml of the concentrated sample preparation to dryness using a stream of nitrogen, dissolve solids in 1 ml 70:30 hexane:ethyl acetate, and add 0.5 ml to HPLC vials. Analyze this sample and the system suitability solution for HPLC using the HPLC conditions above.

Results

Compare the results of the chromatogram from the system suitability solution for HPLC to identify the lutein and zeaxanthin peaks at a resolution of not less than 3.

Calculation

Using the results obtained from the UV/Vis spectrophotometry calculate the % Total carotenoids

$$\% \text{ Total carotenoids} = \frac{\text{Absorbance at 446 nm} \times 10000 \times 100}{\text{sample weight in g} \times 2550}$$

Note: The factors 10000 and 2550 are the dilution factor and extinction value for a 1% solution, respectively.

Using the chromatogram of the sample, calculate the concentration of lutein and zeaxanthin.

$$\text{Lutein (\%)} = \% \text{ Total carotenoids} \times \frac{\text{Peak Area LUTEIN}}{\text{Peak Area TOTAL}}$$

$$\text{Zeaxanthin (\%)} = \% \text{ Total carotenoids} \times \frac{\text{Peak Area ZEAXANTHIN}}{\text{Peak Area TOTAL}}$$

MODIFIED STARCHES

Prepared at the 86th JECFA (2018) and published in FAO JECFA Monograph 22 (2018), superseding specifications included in 16 individual specification monographs prepared at the 82nd JECFA (2016), published in FAO JECFA Monographs 19 (2016).

MODULAR MONOGRAPH consisting of "GENERAL SPECIFICATIONS"^(a) that contains common specifications to all modified starches (INS 1400, 1401, 1402, 1403, 1404, 1405, 1410, 1412, 1413, 1414, 1420, 1422, 1440, 1442, 1450, 1451), and 8 ANNEXES that contain specifications related to the chemical treatments of native starches:

- ANNEX 1^(a) – Fragmentation.
- ANNEX 2^(a) – Bleaching.
- ANNEX 3^(a) – Esterification and/or crosslinking with phosphorous containing compounds.
- ANNEX 4^(a) – Acetylation.
- ANNEX 5^(a) – Oxidation.
- ANNEX 6^(a) – Esterification with octenyl succinic anhydride.
- ANNEX 7^(a) – Etherification with propylene epoxide.
- ANNEX 8^(a) – Esterification and crosslinking with adipic anhydride.

The General specifications are applicable to the following modified starches, each of which should additionally fulfil the specifications of the ANNEXES as follows:

Modified Starch	INS	Annex	ADI	STATUS
Dextrin roasted starch	1400	1	N.S. ⁽¹⁾	T ⁽³⁾
Acid treated starch	1401	1	N.S. ⁽¹⁾	T ⁽³⁾
Alkaline treated starch	1402	1	N.S. ⁽¹⁾	T ⁽³⁾
Bleached starch	1403	2	N.S. ⁽¹⁾	T ⁽³⁾
Oxidized starch	1404	5	N.S. ⁽¹⁾	T ⁽³⁾
Enzyme-treated starch	1405	1	N.S. ⁽¹⁾	T ⁽³⁾
Monostarch phosphate	1410	3	N.S. ⁽¹⁾	T ⁽³⁾
Distarch phosphate	1412	3	N.S. ⁽¹⁾	T ⁽³⁾
Phosphated distarch phosphate	1413	3	N.S. ⁽¹⁾	T ⁽³⁾
Acetylated distarch phosphate	1414	3, 4	N.S. ⁽¹⁾	T ⁽³⁾

Starch acetate	1420	4	N.S. ⁽¹⁾	T ⁽³⁾
Acetylated distarch adipate	1422	4, 8	N.S. ⁽¹⁾	T ⁽³⁾
Hydroxypropyl starch	1440	7	N.S. ⁽¹⁾	T ⁽³⁾
Hydroxypropyl distarch phosphate	1442	3, 7	N.S. ⁽¹⁾	T ⁽³⁾
Starch sodium octenylsuccinate	1450	6	N.S. ⁽¹⁾	T ⁽³⁾
Acetylated oxidized starch	1451	4, 5	N.S. ⁽²⁾	T ⁽³⁾

Should any of the modified starches be subjected to additional chemical treatment, the appropriate specifications outlined in the respective ANNEX should be met. Consequently, for all fragmented and/or bleached starches the specifications of ANNEXES 1 and/or 2 respectively should be met.

^(a)Prepared at the 86th JECFA (2018) and published in FAO JECFA Monograph 22 (2018), superseding specifications included in specification monographs prepared at the 82nd JECFA (2016), published in FAO JECFA Monographs 19(2016).

⁽¹⁾An ADI “not specified” was established at the 26th JECFA (1982).

⁽²⁾An ADI “not specified” was established at the 57th JECFA (2001).

⁽³⁾ T: TENTATIVE

Summary Table

GENERAL REQUIREMENTS IDENTIFICATION					PURITY			
Solubility	Microscopy	Iodine Stain	Copper Reduction	pH	Loss on Drying	Lead	Microbiological Criteria	Sulfur dioxide
Insoluble in cold water	Granular structure typical of the starch source	Colour from dark blue to red after addition of tri-iodide	Red precipitate after addition of hot alkaline cupric tartrate to a test sample refluxed under acidic condition	3.0 -9.0	Cereal starch ≤15.0%; Potato starch: ≤21.0%; Other starches: ≤18.0%	≤0.2mg/kg d.w. Pb (≤0.1 mg/kg) for OSA for infant formula	Aerobic Plate Count: ≤1000 CFU/g; Yeasts and molds: ≤1000 CFU/g; Total Coliforms: ≤10 cfu/g; Information required.	≤50 mg/kg d.w. for modified cereal starches; ≤10 mg/kg d.w. for other modified starches
	SPECIFIC REQUIREMENTS					PURITY		
	Modified Starch	Annex	IDENTIFICATION					
	Dextrin roasted (INS 1400)	1	Dispersion index (Information Required); Reducing sugars (Information Required)			No additional		
Acid treated (INS 1401)	1	Dispersion index (Information Required)			No additional			
Alkaline treated starch (INS 1402)	1	Dispersion index (Information Required); Reducing sugars (Information Required)			No additional			
Bleached (INS 1403)	2	No additional			Carboxyl groups (≤0.1% d.w.);Residual oxidising substances (Information Required)			
Oxidized (INS 1404)	5	hypo-chlorite oxidized starch			Carboxyl groups (≤1.3% d.w.);Residual hypochlorite (Information Required)			
Enzyme-treated (INS1405)	1	Dispersion index (Information Required); Reducing sugars (Information Required)			No additional			
Monostarch phosphate (INS 1410)	3	Information required			Phosphate (≤0.5% d.w. for potato or wheat starches; ≤0.4% d.w. for other starches)			
Distarch phosphate (INS 1412)	3	Information required			Phosphate (≤0.5% d.w. for potato or wheat starches; ≤0.4% d.w. for other starches)			
Phosphated distarch phosphate (INS 1413)	3	Information required			Phosphate (≤0.5% d.w. for potato or wheat starches; ≤0.4% d.w. for other starches)			
Acetylated distarch phosphate (INS 1414)	3, 4	Acetyl group; Ester group; Information required			Phosphate (≤0.14% d.w. for potato or wheat starches; ≤0.04% d.w. for other starches) Acetyl groups (≤2.5% d.w.); Ester groups (≤0.5% d.w.)			
Starch acetate (INS 1420)	4	Acetyl group; Ester group			Acetyl groups (≤2.5% d.w.); Ester groups (≤0.5% d.w.)			
Acetylated distarch adipate (INS 1422)	4, 8	Acetyl group; Ester group; Information required			Acetyl groups (≤2.5% d.w.); Vinyl acetate (≤0.1 mg/kg); Ester groups (≤0.5% d.w.) Adipate groups (≤0.135% d.w.); Residual free adipic acid (Information Required)			
Hydroxypropyl starch (INS 1440)	7	Hydroxypropyl ether groups			Hydroxypropyl groups (≤7.0% d.w.); Propylene chlorohydrins (≤1 mg/kg d.w.)			
Hydroxypropyl distarch phosphate (INS 1442)	3, 7	Hydroxypropyl ether groups; Information required			Phosphate (≤0.14% d.w. for potato or wheat starches; ≤0.04% d.w. for other starches) Hydroxypropyl groups (≤7.0% d.w.); Propylene chlorohydrins (≤1 mg/kg d.w.)			
Starch sodium octenylsuccinate (INS 1450)	6	No additional			Octenylsuccinyl groups (≤3% d.w.); Residual free octenylsuccinic acid (≤0.3% d.w.);			
Acetylated oxidized starch (INS 1451)	4, 5	Acetyl group			Acetyl groups (≤2.5% d.w.); Vinyl acetate (≤0.1 mg/kg); Ester groups (≤0.5% d.w.) Carboxyl groups (≤1.3% d.w.);Residual hypochlorite (Information Required)			

GENERAL SPECIFICATIONS FOR MODIFIED STARCHES

(VERSION 2018 - TENTATIVE)

Information required:

- *Suitable microbiological acceptance criteria and supporting data*

DEFINITION

Starch consists mainly of amylose and amylopectin. Amylose is a linear molecule of α -D-glucopyranosyl units linked by (1-4)- α -linkages. Amylopectin is a highly-branched polymer of α -D-glucopyranosyl units linked by (1-4)- α -linkages and by (1-6)- α -linkages that constitute the branch points. Each glucose unit possesses a maximum of three hydroxyls that can undergo chemical substitution.

Native starches can be physically (pre-gelatinized starches) and/or chemically modified for improved functionality. The most common sources of native starch used in these modifications are various roots, tubers, cereals and legumes. Modified starches are used in applications requiring special properties not attainable by native starches.

Chemical modifications of native starches are often performed, in an aqueous suspension under controlled conditions of pH, time and temperature, unless otherwise indicated in the description of the respective annex. After sufficient reaction time, the modified starch is recovered by filtration or centrifugation, washed with water, dried and packaged. The relevant modification reactions can be, separately or in combination, fragmentations (hydrolysis, oxidation, enzymatic), bleaching, oxidation, esterification, etherification or phosphorylation of one or more of the hydroxyl groups of the α -D-glucopyranosyl units or crosslinking using polyfunctional agents.

See the appropriate Annex or Annexes for the treatment that is applicable to individual modified starch products.

C.A.S numbers

See ANNEXES

DESCRIPTION

White or nearly white powder or granules or (if pre-gelatinized) flakes, or amorphous powder or coarse particles.

FUNCTIONAL USES

Thickener, stabilizer, binder, emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in cold water (if not pre-gelatinised); forming typical colloidal solutions with viscous properties in hot water; insoluble in ethanol.

Microscopy Passes test
See description under TESTS

Iodine stain Passes test
See description under TESTS

Copper reduction Passes test
See description under TESTS

PURITY

General Requirements:

pH 3.0 – 9.0
See description under TESTS

Loss on drying (Vol 4) Cereal starch: not more than 15.0%
Potato starch: not more than 21.0%
Other starches: not more than 18.0%
Conditions: 120°, 4 h, vacuum not exceeding 100 mm Hg

Lead (Vol. 4) Not more than 0.2 mg/kg on the dried basis
Not more than 0.1 mg/kg on the dried basis for Starch sodium octenylsuccinate (INS 1450) for use in infant formula and formula for special medical purposes intended for infants(see Annex 6)
Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).

<u>Microbiological Criteria</u> (Vol 4)	Aerobic plate count: Not more than 1000 CFU/g
	Yeasts and moulds: Not more than 1000 CFU/g
	Total coliforms: Not more than 10 CFU/g
	Information required
<u>Sulphur dioxide</u> (Vol. 4)	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

TESTS

IDENTIFICATION TESTS

<u>Microscopy</u>	Each modified starch, which has not been pre-gelatinized, retains its granular structure and can be identified as a starch by microscopic observation. The typical polarization cross is observed when sample is examined with a polarizing microscope, in polarized light under crossed Nicol prisms. Corn starch: Polygonal, rounded or spherical granules up to 35 µm diameter having a circular or several-rayed central cleft. Potato starch: Irregular shaped, ovoid, pear-shaped granules (30-100 µm diameter, occasionally >100 µm); both, the ovoid, the pear-shaped granules and the rounded granules have an eccentric hilum. All granules show clearly visible concentric striations. Tapioca starch: Spherical granules with one truncated side (5-35 µm diameter) usually having a circular or several-rayed central cleft. Wheat starch: large and small granules (10-60 µm diameter). The central hilum and striations are visible and barely visible.
<u>Iodine stain</u>	Add a few drops of 0.1 N potassium triiodide to an aqueous suspension of the sample. The modified starch stains with iodine in the same way as native starches. The colour can range from dark blue to red.
<u>Copper reduction</u>	Place about 2.5 g of the sample previously washed with water, in a boiling flask; add 10 ml of dilute hydrochloric acid (3%) and 70 ml of water; mix, reflux for about three hours and cool. Add 0.5 ml of the resulting solution to 5 ml of hot alkaline cupric tartrate TS. A copious red precipitate is produced.

PURITY TESTS

pH (Vol. 4)

Suspend 20 g of the sample with 80 ml of water, and agitate continuously at a moderate rate for 5 min (In the case of pre-gelatinised starches, 3 g should be suspended in 97 ml of water).

ANNEX 1: ADDITIONAL SPECIFICATIONS FOR STARCHES MODIFIED BY FRAGMENTATION

(VERSION 2018 - TENTATIVE)

Information is required on:

- *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*

APPLIES TO

Dextrin roasted starch (INS No. 1400)

Acid treated starch (INS No. 1401)

Alkaline treated starch (INS No. 1402)

Enzyme-treated starch (INS No. 1405)

All modified starches that are fragmented

SYNONYMS

Modified starch by fragmentation, converted starch, hydrolysed starch.

TREATMENT

The fragmentation of native starch results in products containing polymers with a lower average molecular weight and reduced viscosity. The manufacturing details for the various modified starches by fragmentation in this monograph are described as below:

- *Dextrin roasted starch, INS. 1400*: is manufactured by dry heating or roasting of native starch with hydrochloric acid or ortho-phosphoric acid in heated and/or agitated vessels. The final dextrin roasted starch is obtained by drying.
- *Acid treated starch, INS. 1401* is obtained by treating a slurry or a suspension of native food starch with dilute hydrochloric acid, ortho-phosphoric acid, or sulphuric acid.
- *Alkaline treated starch, INS. 1402* is obtained by treating a suspended solution of native food starches with sodium hydroxide or potassium hydroxide.
- *Enzyme-treated starch, INS 1405* is obtained by treating a suspension of native food starch with one or more food-grade amylolytic-enzymes (e.g., α -amylase (E.C. 3.2.1.1), β -amylase (3.2.1.2), glucoamylase (3.2.1.3), isoamylase (3.2.1.68), pullulanase (E.C. 3.2.1.41)).

The properties of the modified starches by fragmentation vary depending on the source of native starch, reaction conditions (pH, reaction time, reaction temperature, fragmenting reagent etc.) The alteration of native starch allows for applications that require reduced viscosity in hot solutions and/or typically utilise high levels of modified starches.

C.A.S number	9004-53-9 (Dextrins)
	65996-63-6 (Acid-hydrolysed starch)
	68909-37-5 (Acid-hydrolysed amylopectin)
	9005-84-9 (Starch soluble)
	65996-64-7 (Enzyme-hydrolysed starch)
	1001439-91-3 (Enzyme-treated amylopectin).

CHARACTERISTICS

IDENTITY

<u>Dispersion identity</u>	Information required.
<u>Reducing sugars</u>	Information required

TESTS

IDENTIFICATION TESTS

<u>Dispersion test</u>	Information required
<u>Reducing sugars</u>	Information required

ANNEX 2: ADDITIONAL SPECIFICATIONS FOR BLEACHED STARCHES*(VERSION 2018 - TENTATIVE)**Information is required on:*

- *Suitable method(s) for the determination of residual reagents and data on at least 5 representative batches using the method(s).*

APPLIES TO

Bleached starch INS No. 1403

All modified starches that are bleached

TREATMENT

Peracetic acid and/or hydrogen peroxide, or sodium hypochlorite, sodium chlorite, sulfur dioxide, alternative permitted forms of sulphites, potassium permanganate, or ammonium persulfate

Bleaching is performed to improve physical attributes such as colour due to oxidation of traces of pigments such as carotenoids and xanthophylls. The change is essentially in the colour only. Residual reagents are either removed or limited to technically unavoidable levels.

C.A.S number

977075-42-5

and all other modified starches submitted to bleaching

CHARACTERISTICS**PURITY**Manganese (Vol. 4)

Not more than 50 mg/kg on the dried basis

Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under "General Methods, Metallic Impurities").

Residual oxidising substances

Information required

Carboxyl groups (Vol. 4)

Not more than 0.1% on the dried basis applying the correction for phosphate content as outlined in Note 6 of the method for starches esterified with phosphorus containing compounds.

ANNEX 3: ADDITIONAL SPECIFICATIONS FOR STARCHES ESTERIFIED AND/OR CROSSLINKED WITH PHOSPHORUS CONTAINING COMPOUNDS

(VERSION 2018 - TENTATIVE)

Information required on: A suitable method for identification of crosslinking and data on at least 5 representative batches of crosslinked and non-crosslinked starches.

APPLIES TO

Monostarch phosphate (INS No. 1410)

Distarch phosphate (INS No. 1412)

Phosphated distarch phosphate (INS No. 1413)

Acetylated distarch phosphate (INS No. 1414)

Hydroxypropyl distarch phosphate (INS No. 1442)

TREATMENT

The phosphorus containing compounds ortho-phosphoric acid, sodium or potassium ortho-phosphate and sodium tripolyphosphate, can be used for esterification and the sodium trimetaphosphate or phosphorus oxychloride for crosslinking.

- *Monostarch phosphate (INS 1410)* is obtained by esterification/crosslinking of unmodified food starch with ortho-phosphoric acid, or sodium or potassium ortho-phosphate, or sodium tripolyphosphate
- *Distarch phosphate (INS 1412)* is obtained by crosslinking of unmodified food starch with sodium trimetaphosphate or phosphorus oxychloride
- *Phosphated distarch phosphate (INS 1413)* is obtained by esterification/crosslinking of unmodified food starch with sodium trimetaphosphate or phosphorus oxychloride combined with esterification with ortho-phosphoric acid, or sodium or potassium ortho-phosphate, or sodium tripolyphosphate
- *Acetylated distarch phosphate (NS 1414)* is obtained by esterification/crosslinking of unmodified food starch with sodium trimetaphosphate or phosphorus oxychloride combined with esterification with acetic anhydride or vinyl acetate
- *Hydroxypropyl distarch phosphate (INS1442)* is obtained by esterification of unmodified food starch with sodium trimetaphosphate or phosphorus oxychloride combined with etherification by propylene oxide

Phosphorylation results in partial substitution of the 2, 3- or 6- position of the anhydro glucose 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.

C.A.S numbers	Monostarch phosphate (INS 1410) 11120-02-8(Modified starch) 63055-37-8 (Modified amylopectin)
	Distarch phosphate (INS No. 1412) 55963-33-2(Modified starch) 63055-37-8 (Modified amylopectin)
	Phosphated distarch phosphate (INS No. 1413) 11120-02-8(Modified starch) 63055-37-8 (Modified amylopectin)
	Acetylated distarch phosphate (INS No. 1414) 9067-33-8(Modified starch) 68130-14-3(Modified starch) 113894-91-0 (Modified amylopectin)
	Hydroxypropyl distarch phosphate (INS No. 1442) 53124-00-8(Modified starch) 113894-92-1 (Modified amylopectin)

CHARACTERISTICS

PURITY

<u>Phosphate</u> <u>(calculated as</u> <u>phosphorus)</u> (Vol. 4)	For monostarch phosphate (INS No. 1410), distarch phosphate (INS No. 1412), and phosphate distarch phosphate (INS No. 1413) 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 For acetylated distarch phosphate (INS No. 1414) and hydroxypropyl distarch phosphate (INS No. 1442) 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

IDENTITY

<u>Crosslinking</u>	Information Required

ANNEX 4: ADDITIONAL SPECIFICATIONS FOR ACETYLATED STARCHES*Version 2018***APPLIES TO**

Acetylated distarch phosphate (INS No. 1414)

Starch acetate (INS No. 1420)

Acetylated distarch adipate (INS No. 1422)

Acetylated oxidized starch (INS No. 1451)

TREATMENT

This type of modified starch is obtained by esterification with acetic anhydride or vinyl acetate. Acetylation results in substitution of hydroxyl groups with acetyl esters.

- *Acetylated distarch phosphate (INS 1414)* is obtained by esterification/cross-linking of unmodified food starch with sodium trimetaphosphate or phosphorus oxychloride combined with esterification with acetic anhydride or vinyl acetate.
- *Starch acetate (INS 1420)* is obtained by esterification of food starches with acetic anhydride or vinyl acetate
- *Acetylated distarch adipate (INS 1422)* is obtained by esterification of unmodified food starch with acetic anhydride and esterification/cross-linking with adipic anhydride
- *Acetylated oxidized starch (INS 1451)* is obtained by treatment of food starch with sodium hypochlorite followed by esterification with acetic anhydride

C.A.S numbers

Acetylated distarch phosphate (INS No. 1414)

9067-33-8(Modified starch)

68130-14-3(Modified starch)

113894-91-0 (Modified amylopectin)

Starch acetate (INS No. 1420)

9045-28-7 (Modified starch)

Acetylated distarch adipate (INS No. 1422)

63798-35-6(Modified starch)

63055-36-7 (Modified amylopectin)

Acetylated oxidized starch (INS No. 1451)

68187-08-6(Modified starch)

CHARACTERISTICS

IDENTIFICATION

<u>Specific reaction for Acetyl groups</u>	Passes TEST
	See description under TESTS
<u>Ester groups</u>	Passes TEST
	See description under TESTS

PURITY

<u>Acetyl groups</u>	Not more than 2.5% on the dried basis
	See description under TESTS
<u>Vinyl acetate</u>	Not more than 0.1 mg/kg
	See description under TESTS

TEST

IDENTIFICATION TESTS

<u>Specific reaction for acetyl groups</u>	Principle Acetate is liberated upon saponification of acetylated starch and converted to acetone by heating with calcium hydroxide. The acetone thus produced stains blue with o-nitrobenzaldehyde. Procedure Suspend about 10 g of the sample in 25 ml water. Add 20 ml of 0.4 M NaOH. After shaking for 1 h filter the starch off and evaporate the filtrate in an oven at 110°. Dissolve the residue in a few drops of water and transfer to a test tube. Add calcium hydroxide and heat the tube. If the sample is acetylated starch, acetone vapours are produced. These produce a blue colour on a paper strip soaked in a fresh saturated solution of o-nitrobenzaldehyde in 2 M NaOH. The blue colour is more distinct when the original yellow colour of the reagents is removed with 1 drop of a 1 in 10 solution of hydrochloric acid.
<u>Ester groups</u>	The infrared spectrum of a thin film gives a typical absorption band at about 1720 cm ⁻¹ which is an indication

for ester groups. The limit of detection is about 0.5% acetyl groups in the product.

PURITY TESTS

Acetyl groups

Accurately weigh about 5 g of the sample and transfer into a 250 ml conical flask. Suspend in 50 ml of water, add a few drops of phenolphthalein TS, and titrate with 0.1 M sodium hydroxide to a permanent pink end-point. Add 25.0 ml of 0.45 M sodium hydroxide, stopper the flask, and shake vigorously for 30 min, preferably with a mechanical shaker. (NOTE: the temperature should not exceed 30° as some starches may gelatinise). Remove the stopper, wash the stopper and sides of the flask with a few ml of water, and titrate the excess alkali with 0.2 M hydrochloric acid to the disappearance of the pink colour. Record the volume, in ml of 0.2 M hydrochloric acid required as S.

Perform a blank titration on 25.0 ml of 0.45 M sodium hydroxide, and record the volume, in ml, of 0.2 M hydrochloric acid required as B.

$$\text{Acetyl groups \%} = \frac{(B - S) \times M \times 0.043 \times 100}{W}$$

where

M is the molarity of hydrochloric acid solution; and
W is the weight of sample, in grams.

ANNEX 5: ADDITIONAL SPECIFICATIONS FOR STARCHES SUBJECTED TO OXIDATION

(VERSION 2018 - TENTATIVE)

Information is required on:

- *A suitable method for determination of residual hypochlorite and data on at least 5 representative batches using the method.*

APPLIES TO

Oxidized starch (INS No. 1404)

Acetylated oxidized starch (INS No. 1451)

TREATMENT

Sodium hypochlorite is used for oxidation.

- *Oxidized starch (INS 1404)* is obtained by treatment of food starch with sodium hypochlorite.
- *Acetylated oxidized starch (INS 1451)* is obtained by treatment of food starch with sodium hypochlorite followed by esterification with acetic anhydride.

Oxidation involves the deliberate production of carboxyl groups.

C.A.S number

Oxidised starch (INS No. 1404)
65996-62-5 (modified starch)
113894-86-3 (modified amylopectin)

Acetylated oxidised starch (INS No. 1451)
68187-08-6

CHARACTERISTICS

IDENTIFICATION

Test for hypochlorite oxidized starch

Passes test

See description under TESTS

PURITY

Carboxyl groups (Vol. 4)

Not more than 1.3% on the dried basis

Residual hypochlorite

Information required

TESTS

IDENTIFICATION TESTS

Test for hypochlorite oxidized starch

Principle

Because of the carboxyl group content, hypochlorite-oxidized starch has anionic properties. It can be dyed with positively charged dyes such as methylene blue. The test is not suitable for slightly oxidized potato starch due to the presence of phosphate groups.

Procedure

50 mg of the sample are kept in suspension for 5-10 min in 25 ml of a 1% aqueous dye solution and stirred occasionally. After decantation of the excess solution, the starch is washed with distilled water. Microscopic inspection clearly shows colouring, if the sample is hypochlorite-oxidized starch. By this test hypochlorite-oxidized starch is distinguished from native and acid modified starch of the same botanical origin.

ANNEX 6: ADDITIONAL SPECIFICATIONS FOR STARCHES ESTERIFIED WITH OCTENYLSUCCINIC ANHYDRIDE

Version 2018

APPLIES TO	Starch sodium octenylsuccinate (INS No. 1450)
TREATMENT	Octenylsuccinic anhydride can be used for the esterification and either sodium hydroxide or sodium carbonate as a pH buffer for neutralisation.
C.A.S numbers	Starch sodium octenylsuccinate 66829-29-6(Modified starch) 52906-93-1(Modified starch) 125109-81-1 (Modified amylopectin)
CHARACTERISTICS	
PURITY	
<u>Octenylsuccinyl groups</u>	Not more than 3% on the dried basis See description under TESTS
<u>Residual free octenylsuccinic acid</u>	<i>Not more than 0.3% on the dried basis</i> See description under TESTS
PURITY TEST	
<u>Octenylsuccinate groups and residual free octenylsuccinic acid in Starch sodium octenyl succinate</u>	Principle Residual free octenylsuccinic acid in the sample is extracted and determined by HPLC/UV. Total octenylsuccinic content is determined using the same method after hydrolysis of the sample. Octenylsuccinate ester groups on the modified starch are calculated by subtraction of the residual free octenylsuccinic acid from the total. Standard and Reagents Octenylsuccinic anhydride: 2-Octen-1-ylsuccinic anhydride, mixture of <i>cis</i> and <i>trans</i> (>97%) (CAS: 42482-06-4) 0.1 N potassium hydroxide: Weigh 1.4 g of potassium hydroxide, dissolve in water and dilute to 250 ml

0.073 mol/l phosphoric acid:

Dilute 1 ml of phosphoric acid (85%, density 1.686g/cm³) to 200 ml with water.

Preparation of standard solutions

Accurately weigh about 20 mg of octenylsuccinic anhydride, add 10 ml of 0.1N potassium hydroxide, stopper and heat at 80° for 3 hours. After cooling, add 8 ml of 0.073mol/l phosphoric acid and dilute with water to 20 ml. Pipette 2 ml of this solution into a 20 ml volumetric flask and dilute with water. Pipette 1 ml, 2 ml, 5 ml, and 10 ml of the resulting solution into four separate 20-ml volumetric flasks, and dilute each to volume with water to prepare standards of 5 µg/ml, 10 µg/ml, 25 µg/ml and 50 µg/ml respectively.

Preparation of test solution A (for residual octenylsuccinic acid):

Accurately weigh about 0.1 g of sample, add 20 ml of methanol, and shake for 18 hours or more. Centrifuge the mixture at about 3000 rpm for 5 minutes, pipette 10 ml of the supernatant, and evaporate to dryness under vacuum at 40°. Dissolve the residue and dilute with water in a 5 ml volumetric flask.

Preparation of test solution B (for total octenylsuccinic acid):

Accurately weigh about 20 mg of sample, dissolve in 10 mL of 0.1N potassium hydroxide, stopper and heat at 80° for 3 hours. After cooling, add 8 ml of 0.05mol/l phosphoric acid, dilute with water to 20 ml.

Procedure**HPLC operating conditions**

- Column: A octadecylsilanized silica gel column (250 mm x 4.6 mm, 5µm) (L-Column ODS-V CERI or equivalent)
- Column temperature: 40°
- Detector: UV at 205 nm
- Mobile phase: A 1:1 mixture of 0.1% (v/v) phosphoric acid solution / acetonitrile
- Injection volume: 20µl
- Flow rate: Adjust the retention time of the main peak to about 9 minutes.

Inject the test solution A and B and the standard solutions into an HPLC under the same conditions.

Measure the sum of the peak areas of two main peaks of *cis*- and *trans*-2-octenylsuccinic acid for each standard solution, and prepare a standard curve for octenylsuccinic anhydride from the sums obtained and the concentrations of octenylsuccinic anhydride in the standard solutions. Measure the sum of the peak areas of two main peaks for the test solutions A and B. Determine the concentration of

octenylsuccinic acid ($\mu\text{g/ml}$) in the test solutions A and B from the standard curve, and calculate residual and total octenylsuccinic acid, respectively. The value of octenylsuccinate groups in the sample is calculated by the following formula:

Calculation:

$$\begin{aligned} \text{Residual free octenylsuccinic acid \%} \\ = C_{OS} \times 1.086 / W_r \times 100 \end{aligned}$$

$$\text{Total octenylsuccinic acid \%} = C_{OS} \times 1.086 / W_s \times 500$$

Where

- 1.086 is the molecular weight of octenylsuccinic acid divided by the molecular weight of octenylsuccinic anhydride
- C_{OS} is the octenylsuccinic anhydride concentration ($\mu\text{g/ml}$);
- W_r or W_s is the dry-basis weight of the sample (g).

Content (%) of octenylsuccinyl groups =
Content of total octenyl succinic acid – Content of residual octenylsuccinic acid.

ANNEX 7: ADDITIONAL SPECIFICATIONS FOR STARCHES ETHERIFIED WITH PROPYLENE OXIDE

(Version 2018 - TENTATIVE)

Information is required on:

- 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.

APPLIES TO

Hydroxypropyl starch (INS No. 1440)

Hydroxypropyl distarch phosphate (INS No. 1442)

TREATMENT

Propylene oxide is used for etherification.

- *Hydroxypropyl starch (INS No. 1440)* is obtained by etherification of unmodified food starch with propylene oxide.
- *Hydroxypropyl distarch phosphate (INS No. 1442)* is obtained by esterification of unmodified 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.

C.A.S numbers

Hydroxypropyl starch(INS No. 1440)
9049-76-7(Modified starch)
74315-67-6 (Modified amylopectin)

Hydroxypropyl distarch phosphate (INS No. 1442)
53124-00-8(Modified starch)
113894-92-1 (Modified amylopectin)

CHARACTERISTICS

IDENTIFICATION

Hydroxypropyl ether groups

Passes test

See description under TESTS

PURITY

Hydroxypropyl groups

Not more than 7.0% on the dried basis

See description under TESTS

Propylene
chlorohydrins

Not more than 1 mg/kg

See description under TESTS

TESTS**IDENTIFICATION
TESTS**Hydroxypropyl ether
groups²

Ninhydrin reagent

A 3% solution of 1,2,3-triketohydrindene crystals in 5% aqueous sodium bisulfite solution.

Procedure

Weigh 100 mg of the sample into a 100-ml volumetric flask and add 12.5 ml of 2 N sulfuric acid. Prepare a sample of unmodified starch of the same source (i.e. corn or potato) in the same manner. Place the flasks in a boiling water bath and heat until the samples are in solution. Cool and dilute the contents to 100 ml with water. Pipet 1 ml of the solutions into 25-ml graduated test tubes with glass stoppers and, with the tubes immersed in cold water, add dropwise 8 ml of concentrated sulfuric acid to each. Mix well and place the tubes in a boiling water bath for exactly 3 min. Immediately transfer the tubes to an ice bath until the solution is chilled. Add 0.6 ml of ninhydrin reagent, carefully allowing the reagent to run down the walls of the test tubes. Immediately shake well, and place the tubes in a 25° water bath for 100 min. Adjust the volume in each tube to 25 ml with concentrated sulfuric acid and mix by inverting the tubes several times. (Do not shake).

A violet colour develops only in the modified sample within 5 min due to the presence of hydroxypropyl groups (starch ether). For all other non-hydroxypropyl treated starches a light pink colour is observed.

PURITY TESTHydroxypropyl groups

Ninhydrin reagent

A 3% solution of 1,2,3-triketohydrindene crystals in 5% aqueous sodium bisulfite solution.

Procedure

Accurately weigh 50 - 100 mg of the sample into a 100-ml volumetric flask and add 25 ml of 1 N sulfuric acid. Prepare a sample of unmodified starch of the

² USP29-NF34: U.S. Pharmacopeial Convention, Hydroxylpropyl corn starch monograph, 2015. Reproduced from the USP-NF with permission from The U.S. Pharmacopeial Convention (USP)

same source (i.e. corn or potato) in the same manner. Place the flasks in a boiling water bath and heat until the samples are in solution. Cool and dilute the contents to 100 ml with water. If necessary, dilute the sample further to assure the presence of no more than 4 mg of hydroxypropyl group per 100 ml, and then dilute the blank starch in the same proportion. Pipet 1 ml of the solutions into 25-ml graduated test tubes with glass stoppers and, with the tubes immersed in cold water, add dropwise 8 ml of concentrated sulfuric acid to each. Mix well and place the tubes in a boiling water bath for exactly 3 min. Immediately transfer the tubes to an ice bath until the solution is chilled. Add 0.6 ml of ninhydrin reagent, carefully allowing the reagent to run down the walls of the test tubes. Immediately shake well, and place the tubes in a 25° water bath for 100 min. Adjust the volume in each tube to 25 ml with concentrated sulfuric acid and mix by inverting the tubes several times. (Do not shake). Immediately transfer portions of the solutions to 1-cm cells and after exactly 5 min, measure the absorption (A) at 590 nm, using the starch blank as the reference. Prepare a calibration curve with 1-ml aliquots of standard aqueous solutions, containing 10, 20, 30, 40 and 50 µg of propylene glycol per ml.

Calculation

$$\text{Hydroxypropyl groups (\%)} = \frac{C \times 0.7763 \times 10 \times F}{W}$$

where

- C is the amount of propylene glycol in the sample solution read from the calibration curve (µg/ml);
- F is the dilution factor (if a further dilution has been necessary); and
- W is the weight of sample (mg).

Propylene chlorohydrins

Principle

Propylene chlorohydrins (1-chloro-2-propanol and 2-chloro-1-propanol) in sample are determined by capillary gas chromatography.

Standards and Reagents

propylene chlorohydrins

Preparation of standard addition calibration curve

Accurately weigh about 50 mg propylene chlorohydrins, and dilute with water to 100 ml. Dilute 10 ml of this solution to 100 ml with water to make a standard stock solution (50 µg/ml). Take four Erlenmeyer flasks and weigh 50.0 g of sample in each one. Add 125 ml of 1 M sulfuric acid in each one. Add 0.5 ml, 1 ml, 2 ml, or 5 ml of standard stock solution, to the 1st, 2nd, etc flasks respectively. Proceed as directed for the test solution, beginning

with “and swirl the flask to disperse the contents,” to prepare the standard solutions of added concentration 5, 10, 20 and 50 µg/ml respectively.

Preparation of test solution

Accurately weigh 50 g of sample into an Erlenmeyer flask, add 125 ml of 1 M sulfuric acid, and swirl the flask to disperse the contents. Stopper loosely, heat in a water bath at 100° for 10 min, mix the contents well, and heat for an additional 30 min. For starches that are not easy to hydrolyze, such as wheat starch, heating time should be longer (90 min). Cool to room temperature, adjust the pH to 7 with 25% sodium hydroxide solution, and filter with suction through a glass-fiber filter paper. Wash the flask and the residue on the filter paper with 25 ml of water, and combine the washings with the filtrate. Add 30 g of anhydrous sodium sulfate, stir for 5–10 min to dissolve, and transfer the solution into a separating funnel. Wash the flask with 25 ml of water, and add the washings to the funnel. If precipitate remains, stir well with a small amount of water to dissolve it completely, and add the solution to the funnel. Extract five times with five 50 ml portions of diethyl ether. Combine the diethyl ether extracts, add 3 g of anhydrous sodium sulfate, let it stand for a few minutes and filter through a filter paper. Wash the flask and the filter paper with 25 ml of diethyl ether, and combine the washings with the filtrate. Evaporate to 4 ml in a water bath at about 40° under atmospheric pressure, cool, transfer to a 5 ml volumetric flask and add diethyl ether to the exact volume.

Procedure

GC operating conditions

- GC equipped with a flame ionization detector (FID).
- Column: A fused silica column coated with polyethylene glycol (30 m x 0.25 mm i.d., 0.25 µm) (Inert Cap WAXGL Sciences or equivalent)
- Carrier gas: N₂ or He
- Flow rate: Adjust the retention time of 1-chloro-2-propanol to about 15 min
- Column temperature: 40° for 2 min; heat at 5°/min to 80°, keep for 8 min, heat at 25°/min to 230°, keep for 5 min
- Injector temperature: 150°
- Detector temperature: 230°
- Split-less (purge start: 1 min after injection)

Analyse 1- μ l portions of the test solution and the standard solutions by gas chromatography, using the operating conditions given above. Prepare a standard addition curve: Plot in the y axis the sum of the peak areas corresponding to 1-chloro-2-propanol and 2-chloro-1-propanol in the chromatographs and in the x-axis the added concentration of propylene chlorohydrins in the standard solution. For the test solution the added concentration is equal to 0. A linear calibration curve should be obtained. Extrapolate to 0 in the y axis. The concentration (μ g/ml) of propylene chlorohydrins in the test solution is equal to the absolute value of the concentration at the point where the curve intercepts the x axis (C_t). Determine the content of propylene chlorohydrins in the sample using the following formula:

Calculation

$$\text{Content (mg/kg) of Propylene chlorohydrins} = C_t \times 5 / W$$

where

- C_t : amount of propylene chlorohydrins in test solution (μ g/mL);
- W : mass of sample (g, on the dried weight basis)

ANNEX 8: ADDITIONAL SPECIFICATIONS FOR STARCHES CROSSLINKED WITH ADIPIC ANHYDRIDE

(Version 2018 – Tentative)

Information is required on:

- *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*

APPLIES TO

Acetylated distarch adipate (INS No. 1422)

TREATMENT

Adipic anhydride can be used for esterification and crosslinking. 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-(CH₂)₄-CO and starch refers to the linear and/or branched structure.

C.A.S numbers

Acetylated distarch adipate
63798-35-6 (Modified starch)
63055-36-7 (modified amylopectin)

CHARACTERISTICS

PURITY

Adipate groups

Not more than 0.135% on the dried basis

See description under TESTS

Free adipic acid

Information Required

See description under TESTS

PURITY TEST

Adipate groups and free adipic acid

Determine by gas chromatography after derivatization

Principle

Free adipic acid in the sample is extracted and determined by capillary gas chromatography after trimethylsilyl-derivatization. Total adipic acid is determined using the same method after hydrolysis of the sample and adipate groups are calculated by subtraction of the free adipic acid from the total.

Standards and Reagents

Adipic acid (>99%)

Glutaric acid (>99%)

Starch, unmodified (of the same botanical origin as the sample)

Sodium hydroxide solution (4N): weigh 40g of NaOH, dissolve in water and dilute to 250 ml.

Concentrated HCl (36%)

Ethyl acetate

Sodium sulphate, anhydrous

N,O-Bis(trimethylsilyl)trifluoroacetamide

Pyridine

Internal standard solution (1 mg/ml)

Accurately weigh 0.1 g of glutaric acid, dissolve in water and dilute to 100 ml.

Standard stock solution (1 mg/ml)

Accurately weigh 0.1 g of adipic acid, dissolve in 90 ml of warm water, cool to room temperature, dilute to 100 ml and mix.

Working standard solutions (0.02, 0.1, 0.2 and 0.4 mg/ml)

Pipette 1, 5, 10, and 20 ml of the standard stock solution in four separate 50 ml volumetric flasks, and dilute with water.

Procedure

Preparation standard curve solutions

Weigh 1.0 g of starch into each of four Erlenmeyer flasks, add 50 ml of water and 1 ml of internal standard solution. Add 5 ml each of the four working standard solutions, respectively. Stopper the flask and shake them well to disperse the starch, add 50 ml of 4N sodium hydroxide solution, and shake for 5 min. Place the flasks in a water bath, at room temperature, and add cautiously 20 ml of conc. hydrochloric acid. Cool, and quantitatively separately transfer the contents of the flasks into four separation funnels with a little amount of water. Extract three times with 100 ml of ethyl acetate each time. Collect the ethyl acetate layers separately in four dry Erlenmeyer flasks, add 20 g of anhydrous sodium sulphate, allow to stand for 10 min with occasional shaking, and filter into a rotary evaporator flask. Wash the Erlenmeyer flask and the residue on the filter paper twice with a small quantity of ethyl acetate, and combine the washings with the filtrate. Evaporate the ethyl acetate under a reduced pressure of 6.7 kPa at a temperature below 40°. Remove the remaining ethyl acetate completely by nitrogen stream. The evaporation of ethyl acetate should be effected as quickly as possible. Successively add 2 ml of pyridine and 1 ml of N,O-bis(trimethylsilyl)trifluoroacetamide to the residue and stopper the flask. Allow the solution to stand for 1 hour, transfer 2 ml of it into a GC vial, and immediately stopper tightly. Use these solutions to construct standard curve (Internal standard 1 mg/g starch, standards 0.1, 0.5, 1 and 2 mg/g starch respectively)

Preparation of test solution A (for residual free adipic acid)

Weigh accurately about 5 g of sample into an Erlenmeyer flask, add 100 ml of water and 1 ml of the internal standard solution. Shake well for 1 hour, and filter through a 0.45 µm membrane filter. To the filtrate, add exactly 1 ml of hydrochloric acid (in the case of pre-gelatinized starch or water-soluble starch, directly add 1 ml of hydrochloric acid to the resulting suspension without filtering), and transfer into a separation funnel. Proceed as directed for the preparation of standard solutions, beginning with "...and wash the inside of the flask with a little amount of water into the funnel." Use this solution for the determination of residual free adipic acid (Internal standard 1 mg/ 5 g starch).

Preparation of test solution B (for total adipic acid)

Weigh accurately about 1 g of sample into an Erlenmeyer flask, add 50 ml of water and exactly 1 ml of the internal standard solution. Shake the mixture well to disperse the starch, add 50 ml of 4N sodium hydroxide solution and shake well for 5 minutes. Place the flask in a water bath at room temperature, and add cautiously 20 ml of concentrated hydrochloric acid. After cooling, transfer the contents in the flask into a separation funnel. Proceed as directed for the preparation of standard solution, beginning with "...and wash the inside of the flask with a little amount of water into the funnel." Use this solution for the determination of total adipic acid (Internal standard 1 mg/g starch).

ProcedureGC operating conditions

- GC equipped with a flame ionization detector (FID)
Column: A fused silica column coated with a mixture of 50% diphenyl and 50% dimethylpolysiloxane (15 m x 0.25 mm i.d., 0.25 µm)
- Carrier gas: He
- Column flow 1.0 ml/min.
- Column temperature: 120°-5 min-5°/min-150°
(Glutaric and adipic acids elute at about 5 min and 8 min respectively)
- Injector temperature: 250°
- Detector temperature: 250°
- Injection volume 1 µl
- Split ratio: 30:1

Inject standard curve solutions into the capillary GC under the conditions indicated and construct a standard curve using the peak area ratios of adipic acid and glutaric acid against the amounts of adipic acid in the standard solutions (in g). Inject the test solution A and B and obtain the peak area ratio of adipic acid to glutaric acid for each of the test solutions A and B.

Determine the amount of adipic acid in each test solution from the standard curve and calculate the percent of adipate groups using the following formula:

Free adipic acid, %w/w = $[CF/MF] \times 100$

Adipate groups. %w/w = $[CT/MT - CF/MF] \times 100$

where

CT = amount of the total adipic acid in the test solution
B (g)

CF = amount of the free adipic acid in the test solution
A (g)

MT = mass of sample in the test solution for the
determination of total adipic acid (g, on the dried
weight basis)

MF = mass of the sample in the test solution for the
determination of free adipic acid (g, on the dried
weight basis)

NEUTRAL METHACRYLATE COPOLYMER (TENTATIVE)

New tentative specifications prepared at the 86th JECFA (2018) and published in FAO JECFA Monographs 22 (2018). An ADI of “not specified” was established at 86th JECFA (2018).

Information required on:

- *A validated method for the assay of neutral methacrylate copolymer (e.g., quantitative IR)*
- *Performance characteristics (method validation data) of the assay method*
- *Assay and monomers data on at least five batches of products currently available in commerce*

SYNONYMS

E 1206, INS No. 1206, 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.

DEFINITION

Neutral Methacrylate Copolymer is a copolymer comprised of the monomers ethyl acrylate and methyl methacrylate in the molar ratio of 2:1. The copolymer is manufactured by emulsion polymerization of the monomers 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. The copolymer is standardized as a 30% aqueous dispersion with polyethylene glycol monostearyl ether. The copolymer dispersion may contain the residual monomers (methyl methacrylate and ethyl acrylate). Neutral methacrylate copolymer is used as a coating and glazing agent for food supplements and foods for special medical purposes

Chemical name

Poly(ethyl acrylate-co-methylmethacrylate)

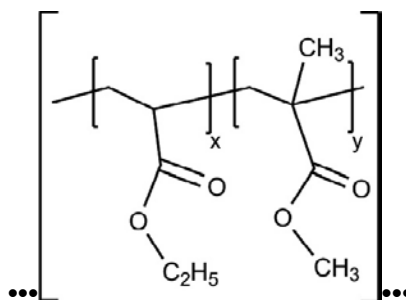
C.A.S. number

9010-88-2

Chemical formula

$\text{Poly}[(\text{CH}_2:\text{CHCO}_2\text{CH}_2\text{CH}_3)\text{-co-}(\text{CH}_2:\text{C}(\text{CH}_3)\text{CO}_2\text{CH}_3)]$

Structural formula



The above formula is provided for illustrative purposes; in this copolymer no definitive structural unit can be defined.

Formula weight 600,000 (weight-average), 220,000 (number-average)

Assay Information required

DESCRIPTION Commercial form (30% aqueous dispersion) is a low viscosity milky-white liquid

FUNCTIONAL USES Coating agent, binding agent, glazing agent

CHARACTERISTICS

IDENTIFICATION

Viscosity (Vol. 4) Not more than 50 mPa.s

Determine viscosity using Brookfield viscometer at 20° and 300 rpm using UL adapter.

pH (Vol 4) 5.5 – 8.6

Infrared absorption

The infrared absorption spectrum of a dry film of sample corresponds to the infrared spectrum in the Appendix.

Apply one drop of sample to a glass plate, cover with a water-resistant crystal disc (AgCl, KRS 5), press lightly, remove the crystal disc and dry for about 15 minutes at 60°.

PURITY

Loss on drying (Vol 4) 68.5 – 71.5% (110°, 3 h)

Sulfated ash (Vol. 4) Not more than 0.4%

Test 5 g of the sample (Method I)

Residual solvents
(Vol. 4)

Methanol: Not more than 100 mg/kg

Residual monomers

Ethanol: Not more than 1,000 mg/kg

Methyl methacrylate: Not more than 50 mg/kg

Ethyl acrylate: Not more than 20 mg/kg

See description under TESTS

Lead (Vol. 4)

Not more than 1.0 mg/kg

Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under "General Methods, Metallic Impurities").

Microbiological criteria
(Vol. 4)

Total plate count: Not more than 1,000 cfu/g

Yeast and moulds: Not more than 100 cfu/g

Coliforms: Negative in 10 g

TESTS

PURITY TESTS

Residual monomers

Determined by liquid chromatography (Vol. 4)

Standards and Reagents:

- Acetonitrile: UV absorption: $A_{\max}$ of 1% at 190 nm
- Tetrahydrofuran and deionized water:
- Sodium perchlorate (35g/l)
- Standards: Ethyl acrylate and methyl methacrylate (>99%)

Preparation of mixed standard solutions

Stock mixed standard solution (200 µg/ml):

Accurately weigh about 10 mg each of ethyl acrylate and methyl methacrylate, dissolve in tetrahydrofuran and make up to 50 ml with tetrahydrofuran in a volumetric flask.

Intermediate mixed standard solution-1 (20 µg/ml):

Dilute 1.0 ml of stock mixed standard solution to 10 ml with tetrahydrofuran in a volumetric flask.

Intermediate mixed standard solution-2 (2 µg/ml):

Dilute 1.0 ml of intermediate mixed standard solution-1 to 10 ml with tetrahydrofuran in a volumetric flask.

Working mixed standard solution (0.67 µg/ml):

To 10 ml of Intermediate mixed standard solution-2 add 5 ml of sodium perchlorate and mix. Dilute 5 ml of this mixture to 10 ml with deionized water.

Preparation Sample Solution

Accurately weigh approximately 1.0 g of sample, dissolve in tetrahydrofuran and dilute to 50.0 ml in a volumetric flask. To 5 ml of sodium perchlorate solution, add 10 ml of sample solution drop wise, whilst stirring continuously. Centrifuge and filter the clear supernatant. Dilute 5 ml of this mixture to 10 ml with deionized water.

Procedure

Use a HPLC with diode array/UV detector at 205 nm

Column: Octadecylsilyl silica gel (12 cm x 4.6 mm i.d., 5-10 µm.)

Injection volume: 50 µl

Mobile phase: Acetonitrile:Water (15:85)

Flow rate: 2 ml/min

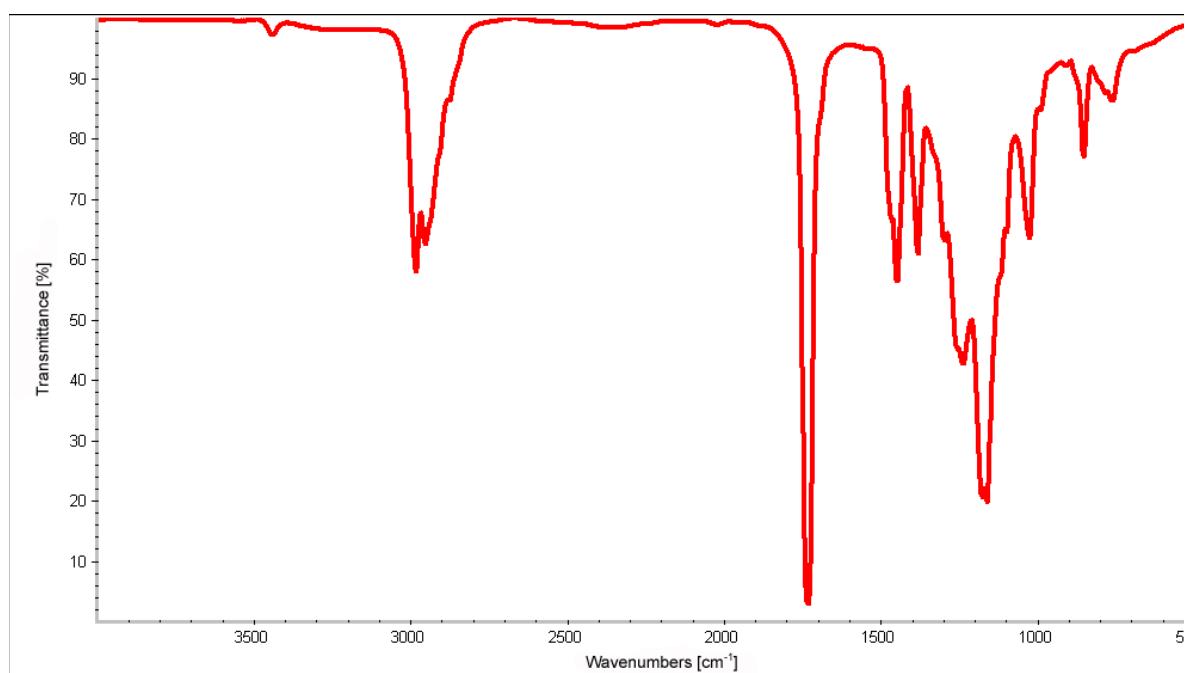
Inject separately 50 µl each of working mixed standard solution and sample solution. Calculate the amount of each monomer in the sample from the peak areas obtained in the chromatograms of working mixed standard solution (rR) and sample solution (rS); amount of standard in the injected solution (R, µg) and weight of sample in injected sample solution (W, g)

$$\text{Amount of each monomer (}\mu\text{g/g)} = \frac{rS \times R}{rR \times W}$$

Total monomers in the sample (µg/g) = Sum of monomers in the sample

METHOD OF ASSAY Information Required

Appendix: Infrared spectrum of neutral methacrylate copolymer



SPIRULINA EXTRACT (TENTATIVE)

New specifications prepared at the 86th JECFA (2018), published in FAO JECFA Monograph 22 (2018). A temporary ADI “not specified” was established at the 86th JECFA (2018).

Information Required on:

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

SYNONYMS

INS 134; Spirulina colour

DEFINITION

Spirulina extract is obtained by aqueous extraction of the biomass of *Arthrospira platensis*, an edible cyanobacterium. The organism is cultivated and harvested under conditions that prevent the growth of other cyanobacteria and the production of microcystins. The material extracted from the biomass is further treated by steps that may include pH adjustment, centrifugation, filtration, concentration, sterilization, drying, and dilution to the desired degree of pigment concentration. The main colouring principles are two phycobiliproteins, C-phycocyanin and allophycocyanin, which are water-soluble pigment-protein complexes where the chromophore is covalently bonded to the protein. Extracts may also contain trace amounts of chlorophyll, beta-carotene, and other carotenoids. Spirulina extract may contain peptides, other proteins, carbohydrates and minerals. Commercial products are formulated in liquid and powder forms.

C.A.S. number

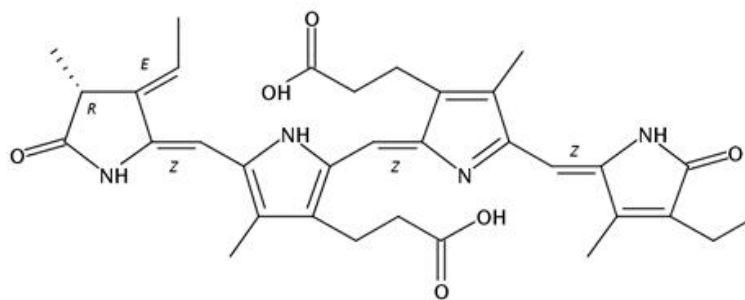
20298-86-6

(Phycocyanobilin; 3-[(2Z,5E)-2-[[3-(2-carboxyethyl)-5-[(Z)-[(3E,4R)-3-ethylidene-4-methyl-5-oxopyrrolidin-2-ylidene]methyl]-4-methyl-1H-pyrrol-2-yl]methylidene]-5-[(4-ethyl-3-methyl-5-oxopyrrol-2-yl)methylidene]-4-methylpyrrol-3-yl]propanoic acid)

Chemical formula

C₃₃H₃₈N₄O₆ (Phycocyanobilin)

Structural formula



Phycocyanobilin

Formula weight 586.68 (Phycocyanobilin)

Assay Total phycocyanins as the sum of C-phycocyanin and allophycocyanin not less than declared.

See description under TESTS

DESCRIPTION Clear blue liquid or blue powder**FUNCTIONAL USES** Colour**CHARACTERISTICS**

IDENTIFICATION

Solubility (Vol. 4) Freely soluble in water. Insoluble in ethanol.Colour Value Not less than declared (15 to 300 for powdered products on the dried basis and 10 to 70 for liquid products).

See description under TESTS

PURITY

Loss on drying (Vol. 4) Not more than 6% for the powdered product (105°, 4h)Arsenic (Vol. 4) Not more than 1 mg/kg

Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Vol. 4 (under "General Methods, Metallic Impurities").

<u>Cadmium</u> (Vol. 4)	Not more than 1 mg/kg
	Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Vol. 4 (under "General Methods, Metallic Impurities").
<u>Lead</u> (Vol. 4)	Not more than 1 mg/kg
	Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Vol. 4 (under "General Methods, Metallic Impurities").
<u>Mercury</u> (Vol. 4)	Not more than 1 mg/kg
	Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Vol. 4 (under "General Methods, Metallic Impurities").
<u>Microbiological criteria</u> (Vol. 4)	Total (aerobic) plate count: less than 1000 CFU/g
	Yeast and moulds: less than 100 CFU/g
	Coliforms: absent in 10 g
	<i>Salmonella spp.</i> : absent in 25 g
	<i>S. aureus</i> : absent in 10 g
<u>Microcystins</u>	Less than 0.5 µg/g as microcystin-LR (dried basis)
	See description under TESTS

TESTS

PURITY TESTS

<u>Microcystins</u>	<u>Principle</u> Determine microcystins by enzyme linked immunoassay (ELISA) under the following conditions:
	<u>Reagent</u> Methanol/water (75:25, v/v)

Equipment

Use a commercially available ELISA kit with cross reactivity for microcystin-LR and other microcystins.

Sample preparation

In the absence of other instructions provided by the ELISA kit manufacturer, follow the procedure presented here.

Dry an appropriate amount of spirulina extract. Homogenize 3.0 g of the dried material in 20.0 ml of the methanol/water reagent for 20 minutes. Centrifuge the resulting suspension at 4500 rpm for 10 minutes. Transfer the supernatant into a glass flask. Add 10.0 ml of the methanol/water reagent to the homogenizer and homogenize the residue for 30 seconds. Centrifuge the resulting suspension at 4500 rpm for 10 minutes. Combine the supernatants and dilute with water to a concentration within the range indicated by the ELISA kit manufacturer.

Procedure

Follow the instructions provided by the ELISA kit manufacturer.

Colour Value

For the purpose of this specification, Colour Value is based on the absorbance of a buffered solution at 618 nm.

Reagent

Sodium phosphate buffer (100 mM, pH 6.0): Transfer 14.04 g of sodium phosphate monobasic dihydrate and 1.75 g of sodium phosphate dibasic anhydrous into a 1000 ml volumetric flask and dilute to volume with water containing 0.05% sodium azide. Adjust the pH to 6.0 with a few drops of phosphoric acid or 1 M NaOH if needed.

Procedure

Transfer 330 mg of spirulina extract into a 100 ml volumetric flask and dilute to volume with water. Transfer 10 ml of the solution into a second 100 ml volumetric flask and dilute to volume with the sodium phosphate buffer (100 mM, pH 6.0). Determine the absorbance (A_{618}) of the solution in a 1-cm cell at 618 nm with a suitable spectrophotometer using sodium phosphate buffer (100 mM, pH 6.0) as the reference.

Calculate the Colour Value of the spirulina extract as follows:

$$\text{Colour Value} = A_{618} \times 100 / W_1$$

Where

W_1 is the weight of spirulina extract taken, in g

METHOD OF ASSAY Principle

Determine total phycocyanins as the sum of C-phycocyanin and allophycocyanin under the following conditions:

Reagent

Sodium phosphate buffer (100 mM, pH 6.0): Transfer 14.04 g of sodium phosphate monobasic dihydrate and 1.75 g of sodium phosphate dibasic anhydrous into a 1000 ml volumetric flask and dilute to volume with water containing 0.05% sodium azide. Adjust the pH to 6.0 with a few drops of phosphoric acid or 1 M NaOH if needed.

Procedure

Transfer 100 mg of spirulina extract into a 25 ml volumetric flask and dilute to volume with sodium phosphate buffer (100 mM, pH 6.0). Sonicate the mixture for 30 minutes maintaining the temperature at 8°. Incubate at 30° for 8 h, shaking manually every hour. Mix the contents of the flask and transfer to a centrifugation tube; centrifuge at 3500 rpm for 4 minutes. Determine the absorbance of the supernatant in a 1-cm cell at 620 nm (A_{620}) and 650 nm (A_{650}) with a suitable spectrophotometer using sodium phosphate buffer (100 mM, pH 6.0) as the reference. The dilution should be adjusted with additional buffer, if needed, to obtain absorbance values of 0.2 to 0.6 at 620 nm.

Calculate the C-phycocyanin content of the spirulina extract (% w/w) as follows:

$$T_{cPC} = (0.162 \times A_{620}) - (0.098 \times A_{650}) \times V_1 \times 100 / W_1$$

Where

W_1 is the weight of spirulina extract taken, in mg

V_1 is the volume of the volumetric flask used to prepare the sample solution, in mL

Calculate the allophycocyanin content of the spirulina extract (% w/w) as follows:

$$T_{aPC} = (0.180 \times A_{620}) - (0.042 \times A_{650}) \times V_1 \times 100 / W_1$$

Where

W_1 is the weight of spirulina extract taken, in mg

V_1 is the volume of the volumetric flask used to prepare the sample solution, in mL

Calculate the total phycocyanin content of the spirulina extract as follows:

$$T_{PC} = T_{cPC} + T_{aPC}$$

SPECIFICATIONS FOR CERTAIN FLAVOURING AGENTS

At the 86th meeting, the Committee prepared specifications of identity and purity of 69 flavourings in 8 sub-categories for the following numbers: 380.1, 380.2, 427, 433, 619, 973-975, 980-982, 1480, 1491-1526, 2103-2105, 2123, 2235-2244, 2246-2255.

Information on specifications for flavouring agents is given in the tables, most of which are self-explanatory: Name; Chemical name (Systematic name, normally IUPAC name); Synonyms; Flavour and Extract Manufacturers' Association of the United States (FEMA) No; FLAVIS (FL) No; Council of Europe (COE) No; Chemical Abstract Service Registry (CAS) No; Chemical formula (Formula); Molecular weight (MW); Physical form/Odour; Solubility; Solubility in ethanol; Boiling point (B.P. °C – for information only); Identification test (ID) referring to type of test (NMR: Nuclear Magnetic Resonance spectrometry; IR: Infrared spectrometry; MS: Mass spectrometry); Assay min % (Gas chromatographic (GC) assay of flavouring agents); Acid value max; Refractive index (R.I.) (at 20°, if not otherwise stated); Specific gravity (S.G) (at 25°, if not otherwise stated). The field called "Other requirements" contains four types of entry:

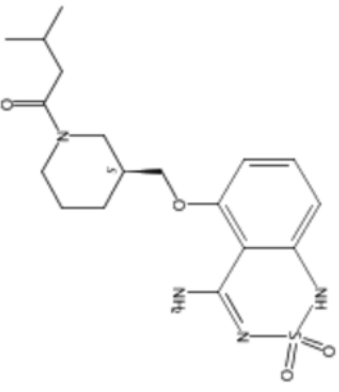
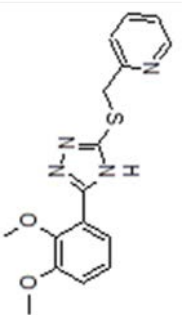
1. Items that are additional requirements, such as further purity criteria or other tests.
2. Items provided for information, for example the typical isomer composition of the flavouring agent. These are not considered to be requirements.
3. Substances which are listed as Secondary Constituents (SC) which have been taken into account in the safety evaluation of the named flavouring agent. If the commercial product contains less than 95% of the named compound, it is a requirement that the major part of the product (i.e. not less than 95% is accounted for by the sum of the named compound and one or more of the secondary constituents.
4. Information on the status of the safety evaluation.

The fields named Session/Status contain the number of the meeting at which the specifications were prepared and the status of the specification. All specifications prepared at the 86th meeting were assigned full status.

The flavouring agents were evaluated using the Procedure for the Safety Evaluation of Flavouring Agents and a list for conclusions in alphabetical order is given in Annex I.

GROUP 1: MISCELLANEOUS NITROGEN-CONTAINING SUBSTANCES

JECFA No.	Name	FEMA	Chemical Formula	Solubility	ID test	R.I. (20°)	Other requirements
Status	Chemical Name	FLAVIS	M.W	Solubility in ethanol	Assay min %	S.G. (25°)	Information required
Synonyms		CE	Physical form; Odour	B.P. °	Acid value		
Session		CAS					
2235	<u>2-(((3-(2,3-Dimethoxyphenyl)-1H-1,2,4-triazol-5-yl)thio)methyl)pyridine</u> Pyridine, 2-(((3-(2,3-dimethoxyphenyl)-1H-1,2,4-triazol-5-yl)thio)methyl) 2-((5-(2,3-dimethoxyphenyl)-2H-1,2,4-triazol-3-ylthio)methyl)pyridine	4798	C ₁₆ H ₁₆ N ₄ O ₂ S	Phosphate buffer, pH 7.1 ± 0.30 mM	MS, 1H-NMR, 13C-NMR, IR	NA	mp: 114.0 - 116.0 °
Draft		328.39	Soluble	>98 %	NA		
Write to off-white solid							
86	<u>(S)-1-(3-((4-Amino-2,2-dioxido-1H-benzol[c][1,2,6]thiadiazin-5-yl)oxymethyl)piperidin-1-yl)-3-methylbutan-1-one</u> (S)-1-(3-(((4-amino-2,2-dioxido-1H-benzol[c][1,2,6]thiadiazin-5-yl)oxymethyl)piperidin-1-yl)-3-methylbutan-1-one	4802	C ₁₈ H ₂₆ N ₄ O ₅ S	Soluble	MS, 1H-NMR, 13C-NMR, IR	NA	mp: 236-238 °, <5% R-enantiomer
Draft		394.49	Soluble	>95 %	NA		
Off-white solid							



mp: 115-
116.5 °C

NA

NA

MS, ¹H-NMR,
¹³C-NMR, IR

Slightly soluble at
pH 2.8

99%

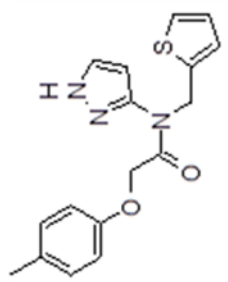
Soluble

C₁₇H₁₇N₃O₂S

327.40

White to off-white solid

2-(4-Methylphenoxy)-N-(1H-pyrazol-3-yl)-N-(thiophen-2-ylmethyl)acetamide
N-(1H-pyrazol-3-yl)-N-(thiophen-2-ylmethyl)-2-(4-tolylloxy)acetamide



2237

Draft

86

1374760-95-8

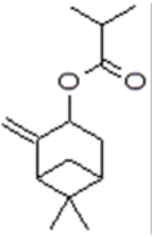
GROUP 2: SATURATED ALIPHATIC ACYCLIC BRANCHED-CHAIN PRIMARY ALCOHOLS, ALDEHYDES, AND ACIDS

JECFA No.	Name	FEMA	Chemical Formula	Solubility	ID test	R.I. (20°)	Other requirements
Status	Chemical Name	FLAVIS	M.W	Solubility in ethanol	Assay min %	S.G. (25°)	Information required
Session	Synonyms	CE	Physical form; Odour	B.P. °	Acid value		
		CAS					
2238	<u>8-Methyldecanal</u>	4795	C ₁₁ H ₂₂ O	very slightly soluble	MS, 1H-NMR, 13C-NMR	1.4224-1.4421	
Draft	8-methyldecanal	170.29	colorless liquid/ citrus/green odor	Soluble	95%	0.879-0.919 (20 °)	
				222-223 °	10		
86		127793-88-8					
2239	<u>8-Methylnonanal</u>	4803	C ₁₀ H ₂₀ O	very slightly soluble	MS, 1H-NMR, 13C-NMR	1.4256 - 1.4260	
Draft	8-Methylnonanal	156.27	Colorless, transparent liquid/Sweet aroma of fruit with green notes	Soluble	>95%	0.8227 - 0.8231 (20 °)	
	Isodecanal, Isodecaldehyde			196-197 °			
86		3085-26-5					

GROUP 3: LINEAR AND BRANCHED-CHAIN ALIPHATIC, UNSATURATED, UNCONJUGATED ALCOHOLS, ALDEHYDES, ACIDS, AND RELATED ESTERS

JECFA No.	Name	FEMA	Chemical Formula	Solubility in ethanol	ID test	R.I. (20°)	Other requirements
Status	Chemical Name	FLAVIS	M.W		Assay min %	S.G. (25°)	Information required
Session	Synonyms	CE CAS	Physical form; Odour	B.P. °	Acid value		
2240 Draft	<u>trans-6-Octenal</u> (E)-Oct-6-enal (E)-6-Octenal; (6E)-Octenal	4787	C ₈ H ₁₄ O 126.20 Colorless oil / Green, melon-like aroma	Slightly soluble Soluble 171-172°	1H-NMR, 13C-NMR 95%	1.4377 0.8536 (20 °)	
86		63196-63-4					Secondary component: 1-6% 2,6-dimethyl-5-heptenal (No. 349) The sum of 2,6-dimethyl-5-heptenal and 2,6-dimethyl-5-heptenal is ≥ 95%
2241 Draft	<u>2,6-Dimethyl-5-heptenal</u> 2,6-Dimethylhept-5-en-1-ol 2,6-dimethyl-5-hepten-1-ol, Melonal	4789	C ₉ H ₁₈ O 142.24 Colorless to light yellow, transparent liquid/Floral fruity aroma reminiscent of melon	Slightly soluble Soluble 204-206 °	MS, 1H-NMR, 13C-NMR >90%	1.428-1.459 0.794-0.904	
86		4234-93-9					

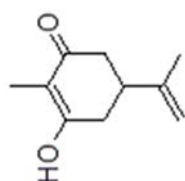
GROUP 4: CARVONE AND STRUCTURALLY RELATED SUBSTANCES

JECFA No.	Name	FEMA	Chemical Formula	Solubility	ID test	R.I. (20°)	Other requirements
Status	Chemical Name	FLAVIS	M.W	Solubility in ethanol	Assay min %	S.G. (25°)	Information required
Synonyms		CE	Physical form; Odour	B.P. °	Acid value		
Session		CAS					
2242	<u>Pinocarvyl isobutyrate</u>	4525	C ₁₄ H ₂₂ O ₂	Practically insoluble to insoluble	MS	1.445-1.450	
Draft	6,6-dimethyl-2-methylidenebicyclo[3.1.1]heptan-3-yl 2-methylpropanoate Propanoic acid, 2-methyl-, 6,6-dimethyl-2-methylenebicyclo[3.1.1]hept-3-yl ester	222.32	Viscous colourless to slight/pale yellow liquid / Warm woody balsamic aroma with fruity notes	Soluble	>95%	0.912-0.915	
86		9291.16-08-5		264-266 °			mp: 144-145 °, Mixture of (2R,4S)-carvyl palmitate and (2S,4S)-carvyl palmitate
2243	<u>Carvyl palmitate</u>	4515	C ₂₆ H ₄₆ O ₂	Practically insoluble to insoluble	MS, 1H-NMR	NA	
Draft	5-Isopropenyl-2-methyl-2-cyclohexen-1-yl palmitate Carvyl hexadecanoate	390.64	Waxy solid/Rich fatty spearmint to caraway aroma	Soluble	>95%	NA	



2244	<u>6-Hydroxycarvone</u>	4523	C ₁₀ H ₁₄ O ₂	Slightly soluble	MS, 1H-NMR, IR	NA	mp: 185 °
Draft	3-hydroxy-2-methyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-one		166.22	Soluble	>95%	NA	

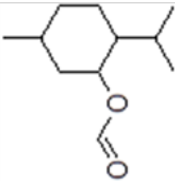
Solid/cool mint-like aroma

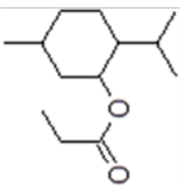


86

51200-86-3

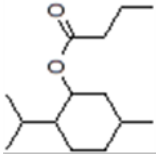
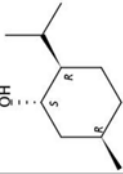
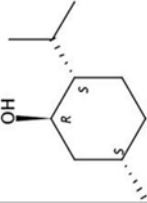
GROUP 5: MENTHOL AND STRUCTURALLY RELATED SUBSTANCES

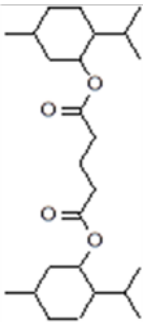
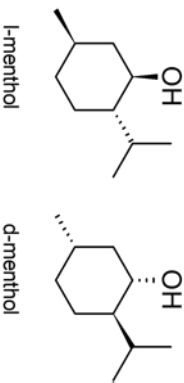
JECFA No.	Name	FEMA	Chemical Formula	Solubility	ID test	R.I. (20°)	Other requirements
Status	Chemical Name	FLAVIS	M.W	Solubility in ethanol	Assay min %	S.G. (25°)	
Synonyms		CE	Physical form; Odour	B.P. °	Acid value		Information required
CAS							
2246	<u>Menthyl formate</u>	4509	C ₁₁ H ₂₀ O ₂	Practically insoluble to insoluble	MS, IR	1.446-1.452	racemic mixture
Draft	2-isopropyl-5-methylcyclohexyl formate		184.28 Clear colorless liquid/Sweet minty aroma	Sparingly soluble 219-220°	>95%	0.933-0.939	
86		2230-90-2					
2247	<u>Menthyl propionate</u>	4510	C ₁₃ H ₂₄ O ₂	Practically insoluble to insoluble	MS	1.444-1.449	
Draft	2-isopropyl-5-methylcyclohexyl propionate		212.34 Clear colorless liquid/fruity cool aroma	Sparingly soluble 246-247 °	>95%	0.918-0.923	



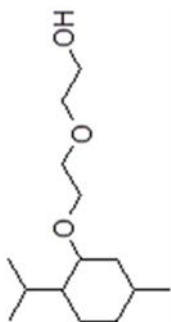
86

86014-82-6

2248	<u>l-Menthyl butyrate</u>	4524	C ₁₄ H ₂₆ O ₂	Practically insoluble to insoluble	MS	1.445-1.450
Draft	2-isopropyl-5-methylcyclohexyl butyrate		226.36 clear colorless liquid/tropical cool aroma	Sparingly soluble	>95%	0.912-0.915
86		68366-64-3		262-263 °		
2249	<u>dl-Isomenthol</u>	4729	C ₁₀ H ₂₀ O	Practically insoluble to insoluble	MS, 1H-NMR, IR	NA
Draft	5-methyl-2-(propan-2-yl)cyclohexan-1-ol		156.27	Sparingly soluble	>95%	NA
	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1- α ,2- β ,5- β)-($\pm$)-; Menthol, trans-1,3,cis-1,4-($\pm$)-; Isomenthol; Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1- α ,2- β ,5- β)-; Isomenthol		Solid/cool minty aroma			
	(1S, 2R, 5R) (+)-Isomenthol					
	(1R, 2S, 5S) (-)-Isomenthol					
86		3623-52-7				

2250	Dimenthyl glutarate	4604	C ₂₅ H ₄₄ O ₄	Practically insoluble to Insoluble	1H-NMR	NA	mp: Approx. 40 °
Draft	bis(2-isopropyl-5-methylcyclohexyl) glutarate	09.935	408.61	Very slightly soluble	>95%	NA	
	Pentanedioic acid, bis[5-methyl-2-(1-methylethyl)cyclohexyl] ester (9CI); Glutaric acid, di-(p-menth-3-yl) Ester		Amber amorphous or crystalline solid/Fresh minty aroma		< 1.0		
86		406179-71-3					
427	Menthol	2665	C ₁₀ H ₂₀ O	very soluble in alcohol and volatile oils; slightly soluble in water	IR	1.461	Melting Range: 41-44° ((-)-menthol); Nonvolatile Residue: =< 0.05%; Angular Rotation: -52° to -40° ((-)-menthol) ; -2° to +2° (dl-menthol)
Full	3-p-Menthanol	156.27			95%; sum of (+/-) isomers	0.901 (20°); 0.891 (30°)	
		63	colourless, hexagonal crystals, usually needle-like; fused masses or crystalline powder with a pleasant, peppermint-like odour	212° ((-)-isomer 216.5)			
51		89-78-1					

2251	<u>(±)-2-[(2-p-Menthoxyl)ethoxyl]ethanol</u>	4718	C ₁₄ H ₂₈ O ₃	Practically insoluble to insoluble	MS, 1H-NMR, IR	1.444 -1.484 (1R, 2S, 5R) (1S, 2R, 5S)
Draft	2-(2-((2-isopropyl-5-methyl)cyclohexyl)oxy)ethoxy)ethan-1-ol		244.37	Soluble	>95% (as a sum of the + and – isomers; racemic mixture)	0.947 - 0.987 (20°); 0.945 -0.985 (25°)
	2-[2-(2-Isopropyl-5-methyl-cyclohexyloxy)-ethoxy]-ethanol		Colorless viscous liquid/Minty herbal aroma with fruity notes	97° (0.2 mmHg)	NA	

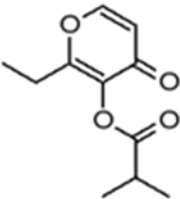


86

28804-53-7

GROUP 6: MALTOL AND RELATED SUBSTANCES

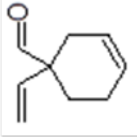
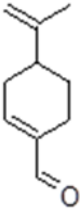
JECFA No. Status	Name Chemical Name Synonyms	FEMA FLAVIS CE CAS	Chemical Formula M.W	Physical form; Odour	Solubility Solubility in ethanol B.P. °	ID test Assay min % Acid value	R.I. (20°) S.G. (25°)	Other requirements Information required
2252 Draft	<u>Ethyl maltol</u> <u>isobutyrate</u> 2-ethyl-4-oxo-4H- pyran-3-yl isobutyrate	4534	C ₁₁ H ₁₄ O ₄		Practically insoluble to insoluble Soluble	MS, IR 93-94%	1.480-1.496 1.132-1.138	Secondary component: 2-3% Ethyl maltol (No. 148
				Clear light yellow liquid/sweet fruity aroma	58-65 ° (2 mm Hg)			



86

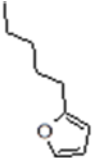
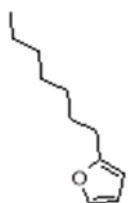
852997-28-5

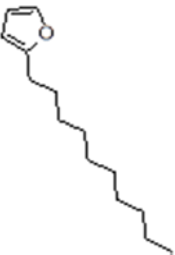
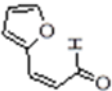
GROUP 7: ALICYCLIC PRIMARY ALCOHOLS, ALDEHYDES, ACIDS AND RELATED ESTERS

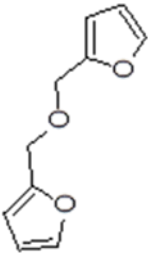
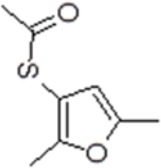
JECFA No.	Name	FEMA	Chemical Formula	Solubility in ethanol	ID test	R.I. (20°)	Other requirements
Status	Chemical Name	FLAVIS	M.W	Solubility in ethanol	Assay min %	S.G. (25°)	
Session	Synonyms	CE	Physical form; Odour	B.P. °	Acid value		Information required
2253	<u>Mixture of 1-Vinyl-3-cyclohexenecarbaldehyde and 4-Vinyl-1-cyclohexenecarbaldehyde</u>	4783	C ₉ H ₁₂ O	Very slightly soluble	MS, 1H-NMR, 13C-NMR	1.4870 - 1.4930	60%-70% 1-vinyl-3-cyclohexenecarbaldehyde and 25-35% 4-vinyl-1-cyclohexenecarbaldehyde
	1-vinylcyclohex-3-ene-1-carbaldehyde and 4-vinylcyclohex-1-ene-1-carbaldehyde						
Draft			136.19 Liquid; Spicy herbal fruit aroma	Soluble	>95% (sum of mixture)	0.9565 - 0.9665	
86		1049017-63-1; 1049017-68-6	1049017-63-1; 1049017-68-6	Insoluble in water; soluble in alcohols and oils	NMR	1.504- 1.513 0.948- 0.956	Safety evaluation not completed
973	<u>p-Mentha-1,8-dien-7-al</u>	3557	C ₁₀ H ₁₄ O	Miscible at room temperature			
Full	p-Mentha-1,8-dien-7-al		150.22		97%		
86	Perilla aldehyde; Dihydrocuminic aldehyde; Perillaldehyde; 4-Isopropenyl-1-cyclohexene-1-carboxaldehyde	11788	Pale, yellowish oily liquid; powerful, fatty-spicy, oily-herbaceous odour	104° (10 mm Hg)	3		
		2111-75-3					

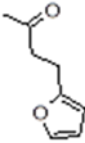
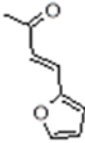
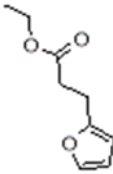
[illegible]

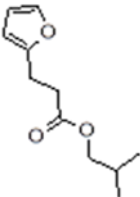
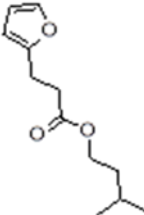
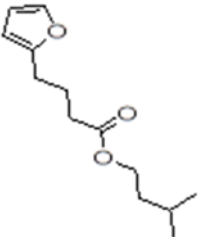
**GROUP 8: FURAN SUBSTITUTED ALIPHATIC HYDROCARBONS, ALCOHOLS, ALDEHYDES,
KETONES, CARBOXYLIC ACIDS AND RELATED ESTERS,
SULFIDES, DISULFIDES AND ETHERS**

JECFA No.	Name	FEMA	Chemical Formula	Solubility in ethanol	ID test	R.I. (20°)	Other requirements
Status	Chemical Name	FLAVIS	M.W		Assay min %	S.G. (25°)	Information required
Session	Synonyms	CE	Physical form; Odour	B.P. °	Acid value		
1491	2-Pentylfuran	3317	C ₉ H ₁₄ O	Slightly soluble in water	NMR	1.443-1.449	
Full	2-Pentylfuran	13.059	138.21	Soluble	99%	0.886-0.893	
	2-Amylfuran		Colourless liquid; Fruity aroma	58-60° (10 mm Hg)	1		
86		3777-69-3					
1492	2-Heptylfuran	3401	C ₁₁ H ₁₈ O	Insoluble in water	NMR	1.446-1.452	
Full	2-Heptylfuran	13.069	166.26 Colourless to yellowish liquid; Nutty, coffee-like aroma	Soluble	99%	0.860-0.866	
86		3777-71-7		209-210°	1		

1493	<u>2-Decylfuran</u>	4090	C ₁₄ H ₂₄ O	Insoluble in water	NMR (13C)	mp: 30°
Full	2-Decylfuran	13.106	208.34	Soluble	95%	
			Colourless solid; Spicy, fatty aroma			
86		83469-85-6				
1494	<u>3-Methyl-2-(3-methylbut-2-enyl)-furan</u>	4174	C ₁₀ H ₁₄ O	Slightly soluble in water	MS	1.473-1.479
Full	3-Methyl-2-(3-methylbut-2-en-1-yl)furan Rosefuran; 2-(3-Methyl-2-butenyl)-3-methylfuran	13.148	150.22	Soluble	98%	0.998-1.004
			Colourless liquid; Caramel aroma	70° (11 mm Hg)	1	
86		15186-51-3				
1497	<u>3-(2-Furyl)acrolein</u>	2494	C ₇ H ₆ O ₂	Insoluble in water	NMR	mp: 49-52°
Full	3-(2-Furyl)prop-2-enal Furylacrolein; 3-(2-Furyl)acrylaldehyde; 3-(2-Furyl)-2-propenal; 2-Furanacrolein	13.034	122.12	Soluble	97%	
			White or yellow needles; Cooked spicy-herb aroma		3	
86		623-30-3				

1522	Difurfuryl ether	3337	C ₁₀ H ₁₀ O ₃	Insoluble in water	NMR	1.138-1.144
Full	Difurfuryl ether	13.061	178.19	Soluble	97%	1.506-1.512
	Furfuryl ether		Colourless to yellow liquid; Coffee-like, nutty aroma	88-89° (1 mm Hg)	1	
86		4437-22-3				
1523	2,5-Dimethyl-3-furanthiol acetate	4034	C ₈ H ₁₀ O ₂ S	Practically insoluble in water and hexane; Soluble in ether	HNMR IR MS	1.527-1.533
Full	2,5-Dimethyl-3-thioacetoxymethoxyfuran	13.116	170.23	Soluble	98%	1.137-1.143
	S-(2,5-Dimethyl-3-furyl) ethanethioate; Thioacetic acid S-(2,5- dimethyl-furan-3-yl) ester		Colourless liquid; Fruity floral aroma	230°	5	
86		55764-22-2				

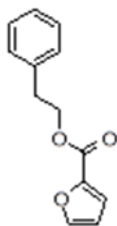
1510	<u>1-(2-Furyl)butan-3-one</u>	4120	C ₈ H ₁₀ O ₂	Slightly soluble in water	MS	mp: 37°
Full	1-(2-Furyl)butan-3-one 1-(2-Furyl)-3-butanone;4-(2-Furyl)-2-butanone;Furfurylacetone	13.138	138.17	Soluble	95%	
						
1511	<u>4-(2-Furyl)-3-buten-2-one</u>	699-17-2	C ₈ H ₈ O ₂	Insoluble in water	NMR	mp: 37-40°
Full	4-(2-Furyl)but-3-en-2-one Furlydene acetone;Furfural acetone	2495 13.044	136.15 Colourless needle crystals; Spicy aroma	Soluble	98% 1	
						
1513	<u>Ethyl 3-(2-furyl)propanoate</u>	623-15-4	C ₉ H ₁₂ O ₃	Very slightly soluble in water	NMR	mp: 24-25°
Full	Ethyl 3(2-furyl)propionate Ethyl furylacetate;Ethyl furiylpropionate;Ethyl 2-furanpropionate	2435 13.022	168.19 Low melting solid, turning yellow on exposure to air; Fruity aroma	Soluble	95% 5	
		10031-90-0				
86						

1514	<u>Isobutyl 3-(2-furan)propionate</u>	2198	C ₁₁ H ₁₆ O ₃	Very slightly soluble in water	NMR	1.531-1.537
Full	2-Methylpropyl 3-(2-furyl)propanoate Isobutyl 2-furanpropanoate;isobutyl furylpropanoate;isobutyl 3-(2- furyl)propanoate	13.024	196.25 Colourless to pale, straw- yellow liquid; Fruity, winey, brandy-like aroma	Soluble	96%	1.007-1.013
86		105-01-1		105° (3 mm Hg)	5	
1515	<u>Isoamyl 3-(2-furan)propionate</u>	2071	C ₁₂ H ₁₈ O ₃	Insoluble in water	NMR	1.549-1.557
Full	3-Methylbutyl 3-(2-furan)propanoate 2-Isoamyl furfurylacetate;3-Methylbutyl 3(2- furyl)propanoate;isoamyl 2-furanpropanoate	13.023	210.27 Colourless to pale yellow liquid; Sweet, green, slightly floral aroma	Soluble	96%	0.987-0.993
86		7779-67-1		258°	5	
1516	<u>Isoamyl 4-(2-furan)butyrate</u>	2070	C ₁₃ H ₂₀ O ₃	Insoluble in water	NMR	1.551-1.555
Full	3-Methylbutyl 4-(2-furan)butanoate Isopentyl 2-furanbutyrate;alpha-Isoamyl furfurylpropanoate;3-Methylbutyl 2- furanbutyrate	13.021	224.3 Pale yellowish liquid; Sweet- buttery, fruity and caramel-like aroma	Soluble	95%	0.975-0.981
86		7779-66-0		263-265°	5	

Phenethyl 2-furoate

Full Phenethyl 2-furoate

2-Phenylethyl 2-furoate; Phenylethyl 2-furoate



86

7149-32-8

Insoluble in water;
Soluble in oils

275°

5

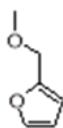
1.585-1.593
1.136-1.142

NMR
96%

Furfuryl methyl ether

Full Furfuryl methyl ether

Methyl furfuryl ether



86

13679-46-4

Insoluble in water;
Soluble in ether

Soluble

134-135°

1

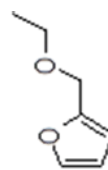
1.454-1.460
1.013-1.019

NMR
99%

Ethyl furfuryl ether

Full Ethyl furfuryl ether

Furfuryl ethyl ether



86

6270-56-0

Slightly soluble in
water

Soluble

150°

1

1.449-1.455
0.982-0.988

MS
95%

C₁₃H₁₂O₃

216.24

Colourless liquid; Warm,
fruity-caramel, slightly earthy,
oily aroma

C₆H₈O₂

112.13

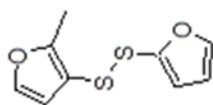
Clear to yellow liquid; Airy,
roasted coffee aroma

C₇H₁₀O₂

126.15

Colourless liquid; Sweet, spicy
aroma

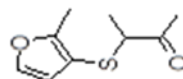
1524
Full
Furfuryl 2-methyl-3-furyl disulfide
Furfuryl 2-methyl-3-furyl disulfide
3-[2-Furanylmethyl)dithio]-2-methylfuran;(2-Methyl-3-furyl)furfuryl disulfide;2-Methyl-3-[(2-furanylmethyl)-dithio]furan;3-(Furfuryldithio)-2-ethylfuran



86

109537-55-5

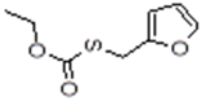
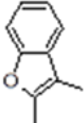
1525
Full
3-[(2-Methyl-3-furyl)thio]-2-butanone
3-[(2-Methyl-3-furyl)thio]-2-butanone
3-[(2-Methyl-3-furanyl)sulfanyl]-2-butanone;3-(2-Methyl-3-furylthio)-2-butanone;3-[(2-Methyl-3-furyl)sulfanyl]-2-butanone



86

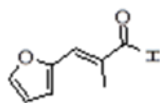
61295-44-1

4119	C ₁₀ H ₁₀ O ₂ S ₂ 226.32	Colourless liquid; Strong, sulfurous aroma	294°	3	Slightly soluble in water; Soluble in pentane, diethyl ether Soluble	HNMR IR 90%	1.581-1.587 1.277-1.283	SC: 6-7% Di-(2-methyl-3-furyl) disulfide
4056 13.190	C ₉ H ₁₂ O ₂ S 184.25	Colourless liquid; Spicy, floral aroma	70° (0.75 mm Hg)	1	Soluble in ethyl acetate, triacetin, Practically insoluble in water Soluble	HNMR MS 99%	1.510-1.516 1.104-1.110	

1526	<u>O-Ethyl S-(2-furylmethyl)thiocarbonate</u>	4043	C ₈ H ₁₀ O ₃ S	186.23	Practically insoluble in water; Soluble in diether ether, ethyl acetate	HNMR IR MS	1.504-1.510
Full	O-Ethyl S-(2-furylmethyl)thiocarbonate				Soluble	99%	1.167-1.173
	O-Ethyl S-(2-furanylmethyl)thiocarbonate; Ethoxycarbonyl furfurylthiol; O-Ethyl S-(furan-2-ylmethyl) thiocarbonate		Colourless liquid; Spicy, floral aroma		130-135°	5	
86		376595-42-5					
1495	<u>2,3-Dimethylbenzofuran</u>	3535	C ₁₀ H ₁₀ O	146.19	Insoluble in water, Soluble in fats	NMR	1.554-1.563
Full	2,3-Dimethylbenzofuran	13.074	Clear to yellow liquid; Nutty spicy aroma	96-98° (15 mm Hg)	Soluble	97%	1.031-1.037
						1	
86		3782-00-1					
1496	<u>2,4-Difurfurylfuran</u>	4095	C ₁₄ H ₁₂ O ₃	228.24	Insoluble in water	NMR (13C)	mp: 153°
Full	2,4-Difurfurylfuran	13.107	Colourless solid; Floral, fruity aroma		Soluble	95%	
86		64280-32-6					

1498 **2-Methyl-3-(2-furyl)acrolein**

Full 3-(2-Furyl)-2-methylprop-2-enal
2-Methyl-3-(2-furyl)propenal; alpha-Methylfurylacroleine; Furfurylidene-2-propanal

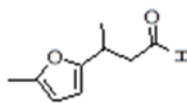


86

874-66-8

1500 **3-(5-Methyl-2-furyl)-butanal**

Full 3-(5-Methyl-2-furyl) butanal
3-(5-Methyl-2-furyl)-butyraldehyde

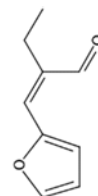


86

31704-80-0

1501 **2-Furfurylidenebutyraldehyde**

Full Furfurylidene-2-butanal
3(2-Furyl)-2-ethylacrolein; 2-Ethyl-3(2-furyl)-2-propenal; 2-Ethyl-3(2-furyl)acrolein



86

770-27-4

Insoluble in water;
Soluble in oils

NMR 1.567-1.573

96% 1.097-1.103

Pale yellowish liquid; Mild, warm, cinnamon-like aroma

225° 3

Insoluble in water;
Soluble in oils

NMR 1.575-1.581

98% 1.006-1.012

Colourless liquid; Vegetable, fruity aroma

88-91° (12 mm Hg) 3

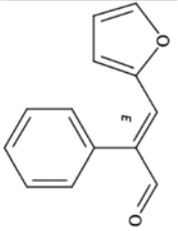
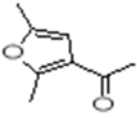
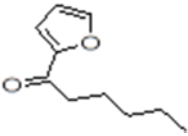
Insoluble in water;
Soluble in oils

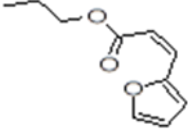
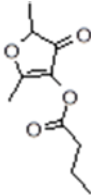
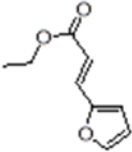
NMR 1.570-1.576

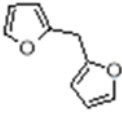
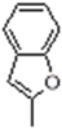
98% 1.057-1.063

Pale yellowish liquid; Mild, warm, vegetable-like aroma

240° 3

1502	<u>2-Phenyl-3-(2-furyl)prop-2-enal</u>	3586	C ₁₃ H ₁₀ O ₂	Insoluble in water	NMR	mp: 56-57°
Full	3-(2-Furyl)-2-phenylprop-2-enal 2-Furfurylidenephenylacetaldehyde	13.137	198.22	Soluble	99%	
86		65545-81-5				
1506	<u>3-Acetyl-2,5-dimethylfuran</u>	3391	C ₈ H ₁₀ O ₂	Slightly soluble in water; Soluble in propylene glycol, most fixed oils	NMR	1.488-1.490
Full	3-Acetyl-2,5-dimethylfuran 2,5-Dimethyl-3-acetylfuran	13.066	138.17	Soluble	99%	1.037-1.039
86		10599-70-9		83° (11 mm Hg)	1	
1512	<u>Pentyl 2-furyl ketone</u>	3418	C ₁₀ H ₁₄ O ₂	Slightly soluble in water	NMR	1.490-1.496
Full	2-Hexanoylfuran 2-Furyl pentyl ketone	13.070	166.22	Soluble	99%	0.992-0.998
86		14360-50-0		65-67° (0.5 mm Hg)	1	

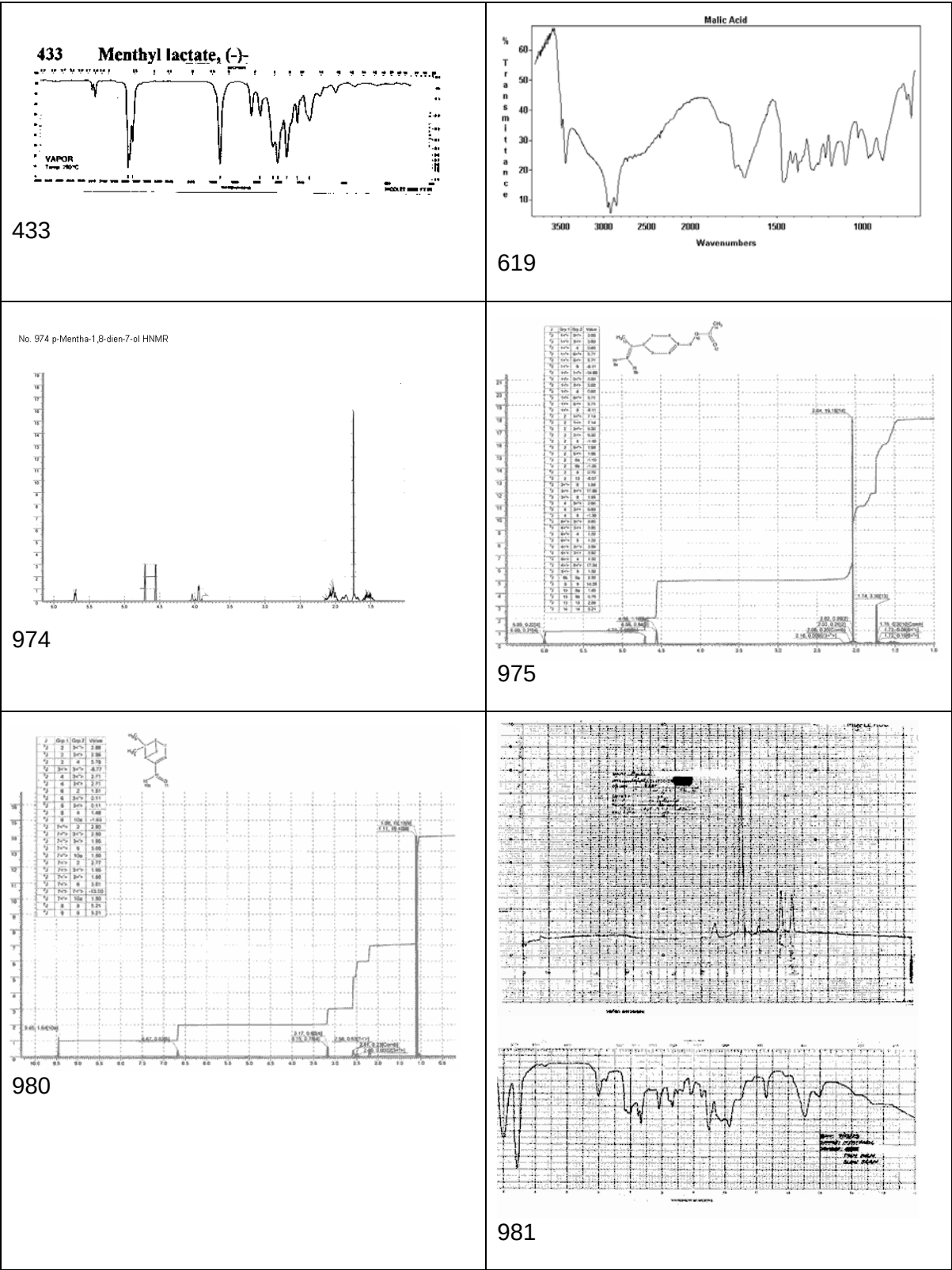
1518	<u>Propyl 2-furanacrylate</u>	2945	C ₁₀ H ₁₂ O ₃	Insoluble in water	NMR	1.071-1.077 (20°)
	Full Propyl 3-(2-furyl)prop-2-enoate 2-Propenoic acid, 3-(2-furanyl)-, propyl ester; Propyl 3-(2-furyl)acrylate	13.047	180.2 Colourless to pale yellow liquid; Light strawberry, pear- like aroma	Soluble 119° (7 mm Hg)	97% 5	
86		623-22-3				
1519	<u>2,5-Dimethyl-3-oxo-(2H)-fur-4-yl butyrate</u>	3970	C ₁₀ H ₁₄ O ₄	Insoluble in water	NMR	1.467-1.473 1.095-1.103
Full	4-Butyryloxy-2,5-dimethyl-3(2H)- furanone		198.22 Colourless to pale yellow liquid; Spicy, sweet aroma	Soluble 287°	93% 5	SC: 1-3% 4- Hydroxy-2,5- dimethyl-3(2H)- furanone and 1- Butyric acid
86		114099-96-6				
2103	<u>(E)-Ethyl 3-(2-furyl)acrylate</u>	4541	C ₉ H ₁₀ O ₃	Practically insoluble to insoluble in water	MS	1.542-1.548 1.090-1.096
Full	Ethyl (2E)-3-(furan-2-yl)prop-2-enoate Ethyl(E)-3-(2-furyl)-2-propenoate		166.17 Viscous liquid; Sweet aroma	Soluble 230-233°	95%	
86		53282-12-5				

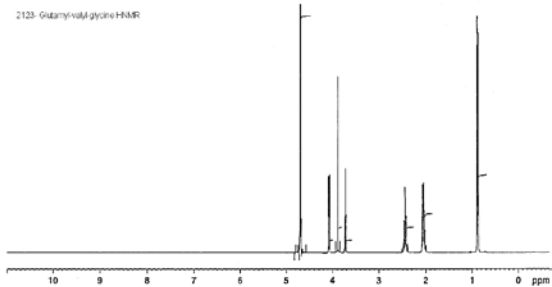
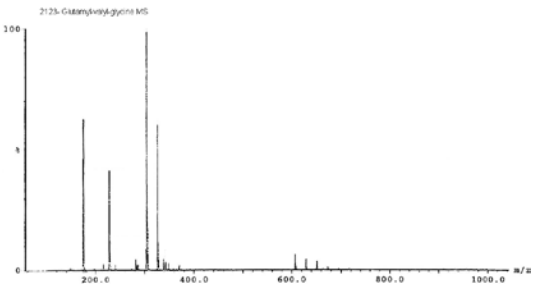
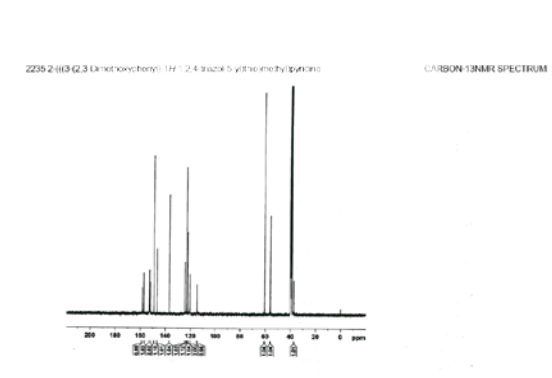
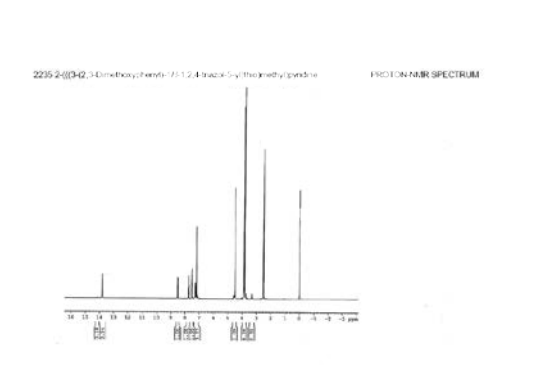
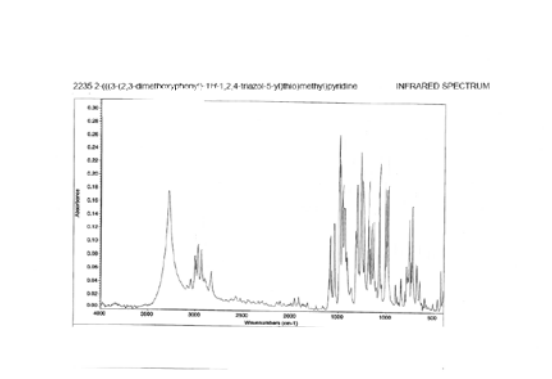
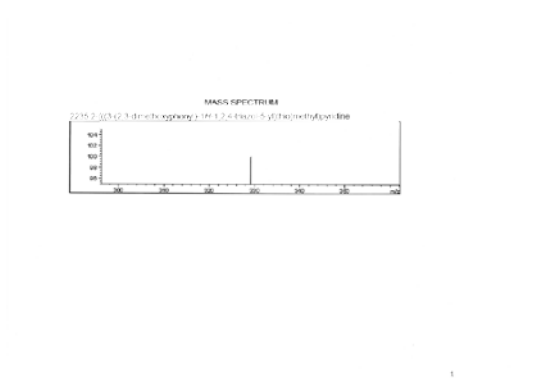
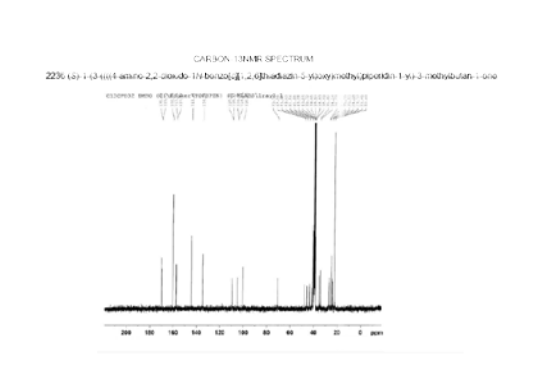
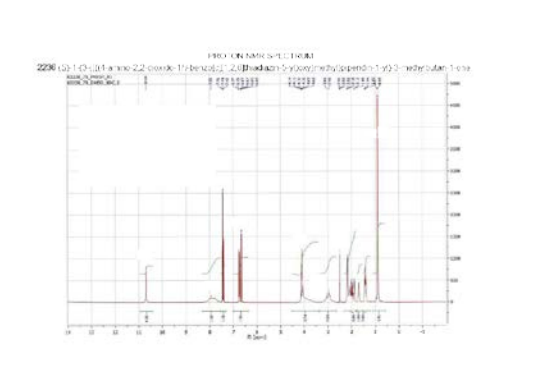
2104	<u>di-2-Furylmethane</u>	4540	C ₉ H ₈ O ₂	Practically insoluble to insoluble in water	MS	1.501-1.507 1.097-1.103 (20°)
Full	2,2'-Methanediyl(difuran di-2-Furyl methane		148.16 Colourless clear liquid; Rich roasted aroma	Soluble 194-195°	95%	
						
86		1197-40-6				
2105	<u>2-Methylbenzofuran</u>	4543	C ₉ H ₈ O	Practically insoluble to insoluble in water	MS	1.548-1.560
Full	2-Methyl-1-benzofuran 2-Methyl benzo(b)furan		132.16 Colourless liquid; Burnt phenolic aroma	Soluble 197-198°	95%	1.052-1.057
						
86		4265-25-2				

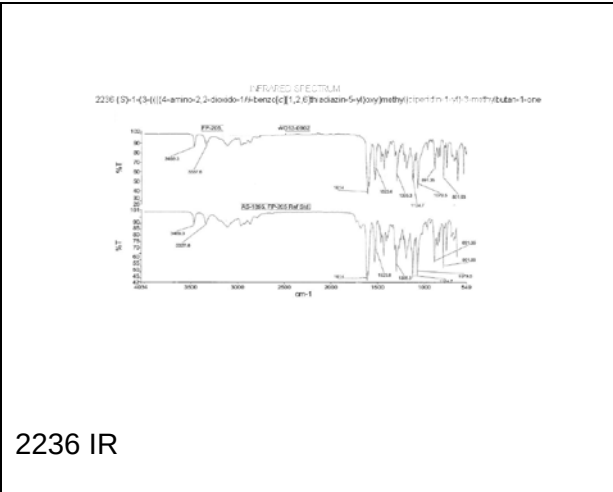
REVISIONS TO EXISTING FLAVOUR SPECIFICATIONS

JECFA No.	Name	FEMA	Chemical Formula	Solubility in ethanol	ID test	R.I. (20°)	Other requirements
Status	Chemical Name	FLAVIS	M.W	Assay min %	Assay min %	S.G. (25°)	
Sessio n	Synonyms	COE	Physical form; Odour	B.P. °	Acid value		Information required
433	<i>L</i> -menthyl <i>L</i> -lactate	CAS 3748	C ₁₃ H ₂₄ O ₃	IR	IR		Melting point c.25°
Full	(1 <i>R</i> ,2 <i>S</i> ,5 <i>R</i>)-2-Isopropyl-5-methylcyclohexyl (2 <i>S</i>)-2-hydroxypropanoate		228.33 colourless liquid or white crystalline solid with a weak chamomile or tobacco odour	97%			
86		61597-98-6		142° (5 mmHg)	2		
619	<i>L</i> -malic acid						Melting point: 100°; Fumaric Acid: max. 1.0%; Heavy Metals: max 10 ppm; Maleic acid: max. 0.05%; Residue on Ignition: max 0.1 %; Water Insoluble Matter: max. 0.1%
Full	2-Hydroxybutanedioic acid	2655	C ₄ H ₆ O ₅ 134.09 White crystalline powder, granules, or needles; Acid taste; odourless or having a very faint caramellic acid odour and a tart acidulous taste	soluble in water and alcohol; 1 gm in 0.8 ml water 1 g in 1.4 ml alcohol	IR 99%		
86		17					
Full	Hydroxysuccinic acid; 2-Hydroxybutanedioic acid						
2123	Glutamyl-valyl-glycine (2 <i>S</i>)-2-Amino-5-(((2 <i>S</i>)-1-[(carboxymethyl)amino]-3-methyl-1-oxobutan-2-yl)amino)-5-oxopentanoic acid	4709	C ₁₂ H ₂₁ N ₃ O ₆ 303.31 Solid off-white powder; Light savoury almost yeastlike aroma	Soluble in water Practically insoluble	MS, HNMR >95%		m.p.: 200-204° (decomposition)
86	L-gamma-glutamyl-L-valyl-glycine	38837-70-6					

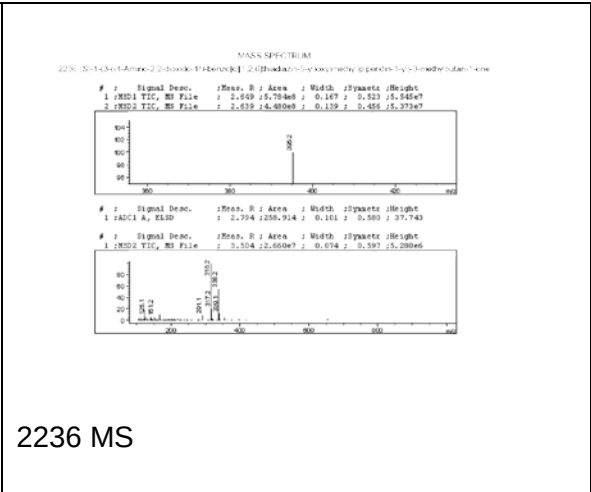
SPECTRA OF CERTAIN FLAVOURING AGENTS



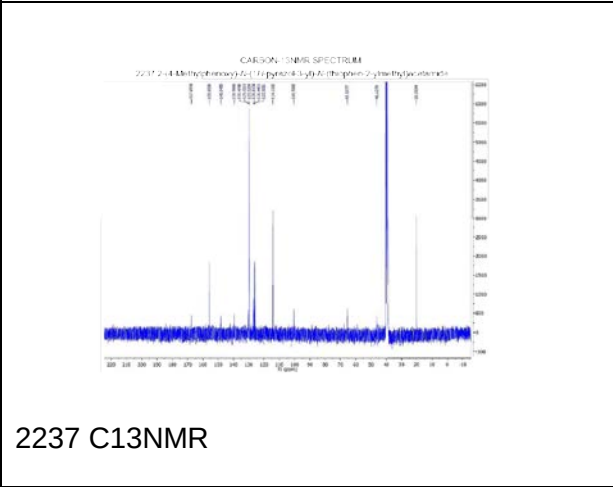
2123- Glutarylvalylglycine HNMR  2123 HNMR	2123- Glutarylvalylglycine MS  2123 MS
2235-2-((3-(2,3-Dimethoxyphenyl)-1H-1,2,4-triazol-5-yl)methyl)pyridine CARBON-13NMR SPECTRUM  2235 C13 NMR	2235-2-((3-(2,3-Dimethoxyphenyl)-1H-1,2,4-triazol-5-yl)methyl)pyridine PROTON NMR SPECTRUM  2235 HNMR
2235-2-((3-(2,3-dimethoxyphenyl)-1H-1,2,4-triazol-5-yl)methyl)pyridine INFRARED SPECTRUM  2235 IR	2235-2-((3-(2,3-dimethoxyphenyl)-1H-1,2,4-triazol-5-yl)methyl)pyridine MASS SPECTRUM  2235 MS
2236-((S)-1-(2-((S)-1-((S)-2,2-dimethyl-1H-benzof[1,2-b]triazol-5-yl)methyl)pyridin-1-yl)-3-methylbutan-1-one) CARBON-13NMR SPECTRUM  2236 C13NMR	2236-((S)-1-(2-((S)-1-((S)-2,2-dimethyl-1H-benzof[1,2-b]triazol-5-yl)methyl)pyridin-1-yl)-3-methylbutan-1-one) PROTON NMR SPECTRUM  2236 HNMR



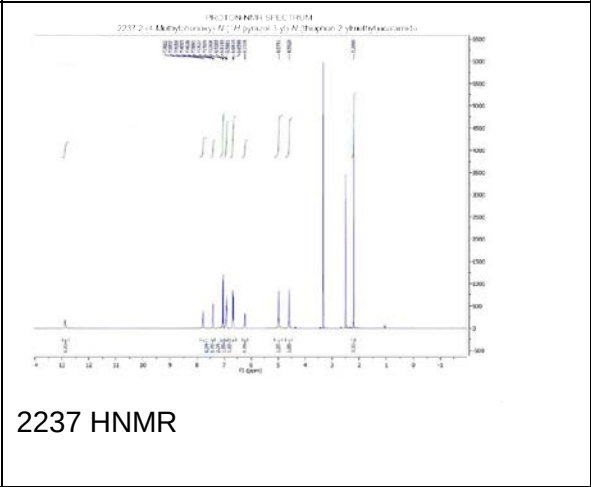
2236 IR



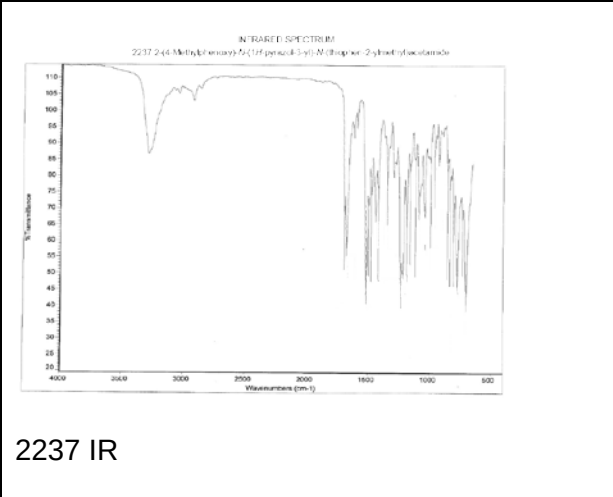
2236 MS



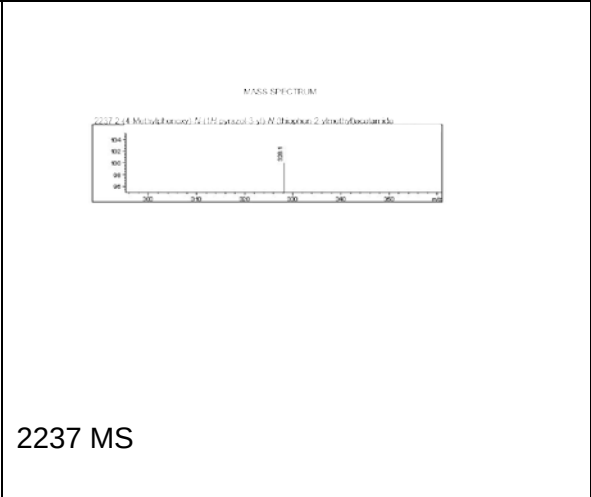
2237 C13NMR



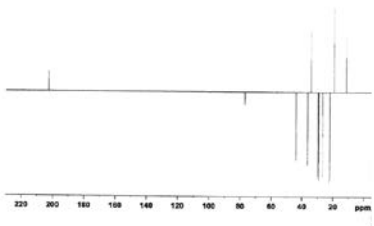
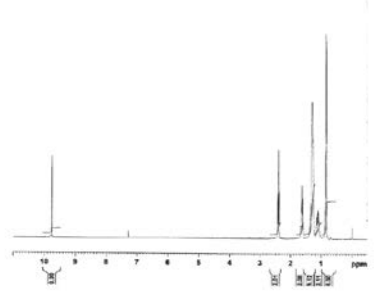
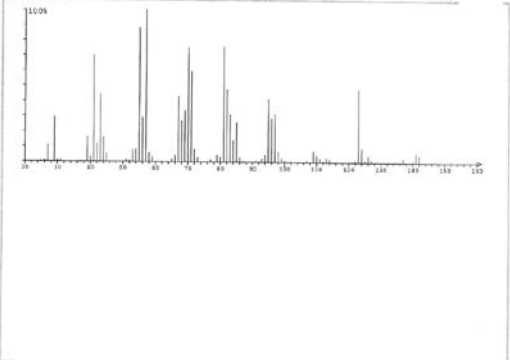
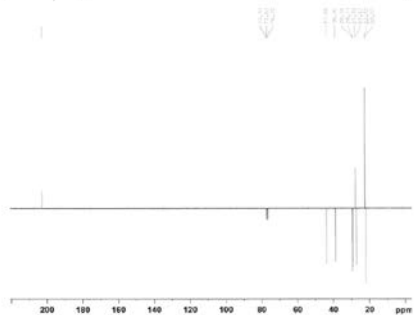
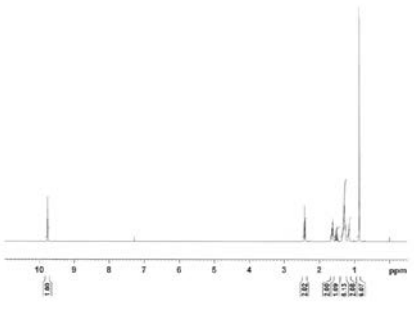
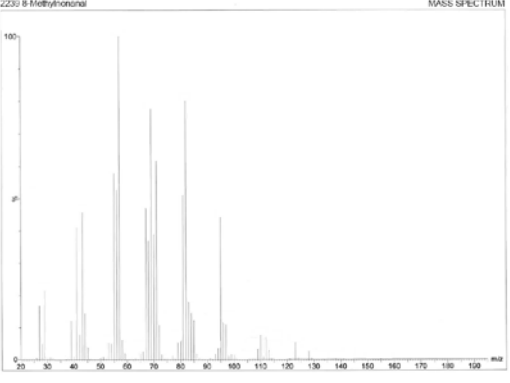
2237 HNMR

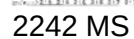
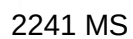
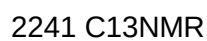
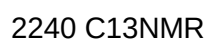


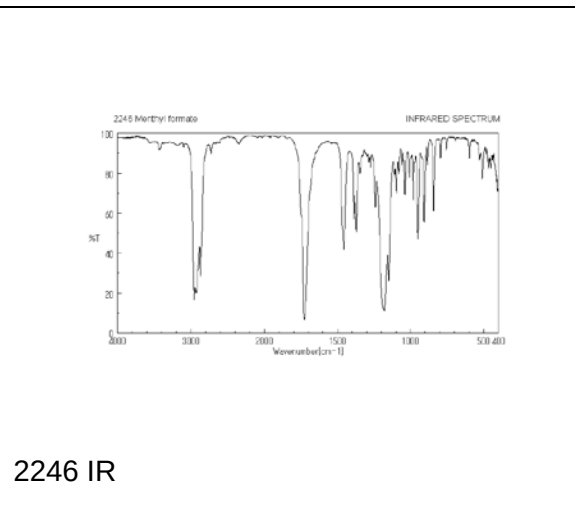
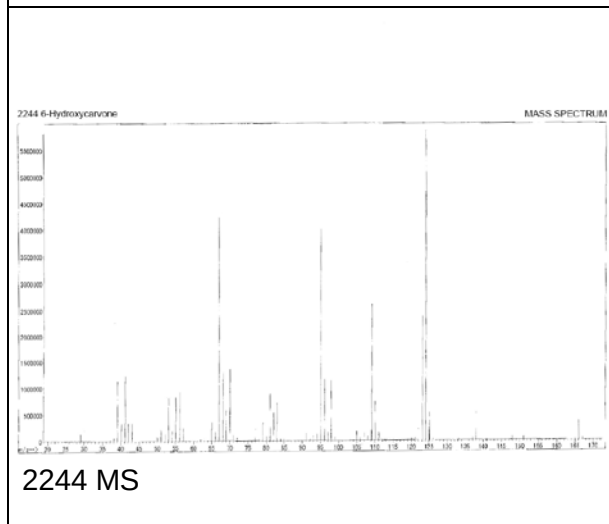
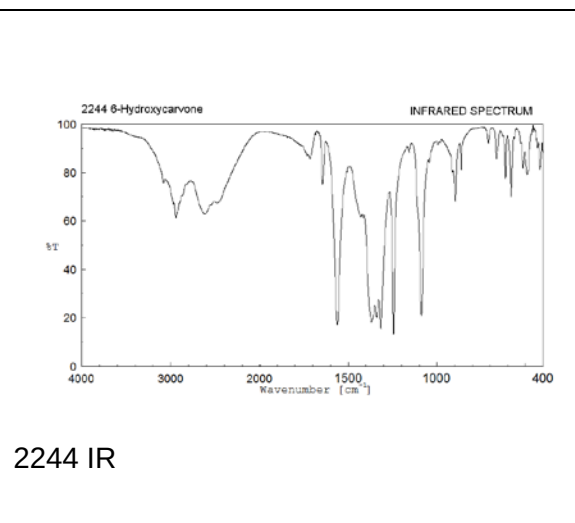
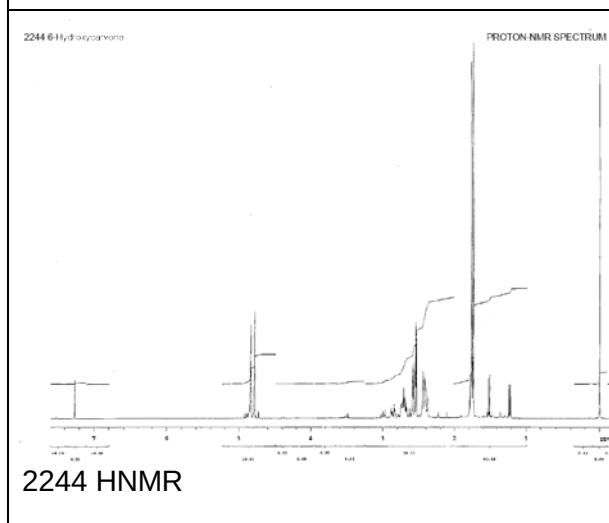
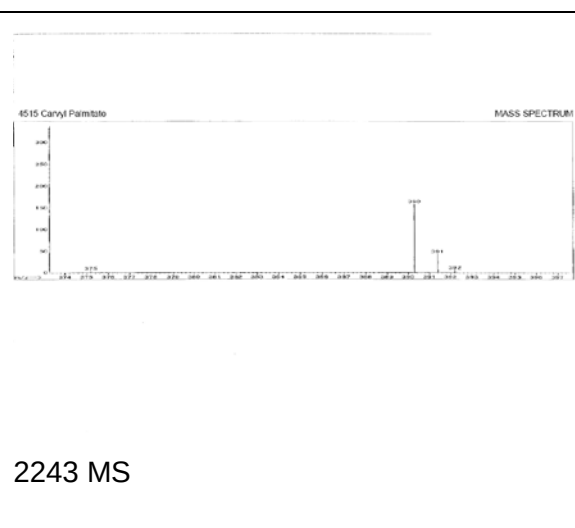
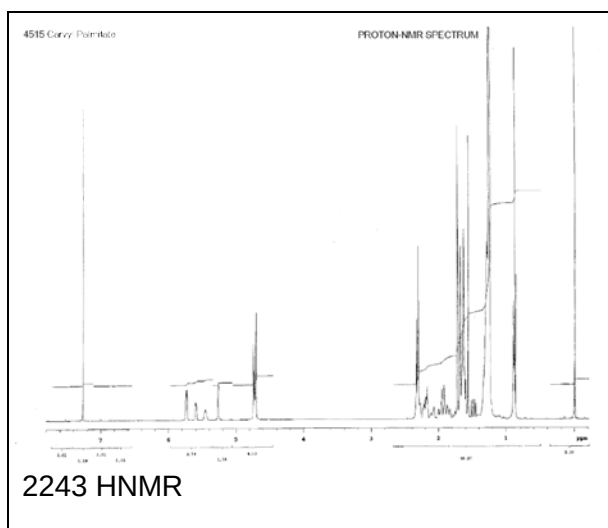
2237 IR

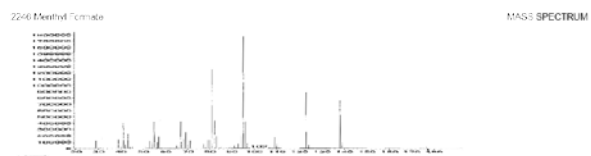


2237 MS

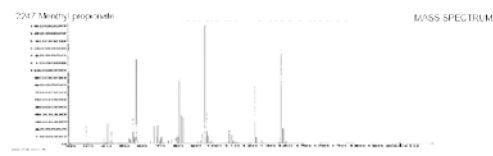
2238 8-Methyldecanal CARBON-13NMR SPECTRUM  2238 C13NMR	2238 8-Methyldecanal PROTON-NMR SPECTRUM  2238 HNMR
2238 8-Methyldecanal MASS SPECTRUM  2238 MS	2239 9-Methylnonanal CARBON-13NMR SPECTRUM  2239 C13NMR
2239 9-Methylnonanal PROTON-NMR SPECTRUM  2239 HNMR	2239 9-Methylnonanal MASS SPECTRUM  2239 MS



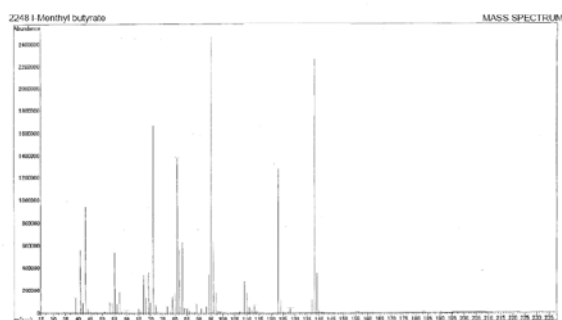




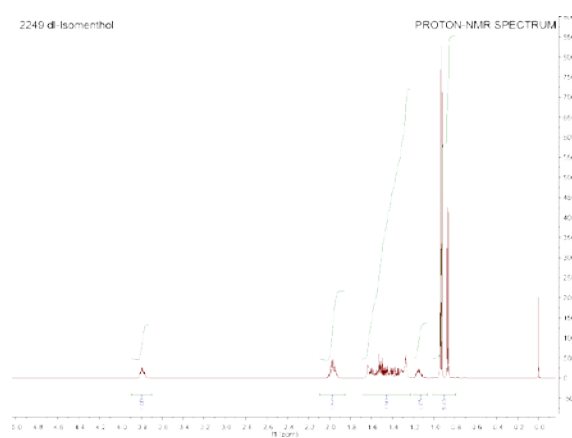
2246 MS



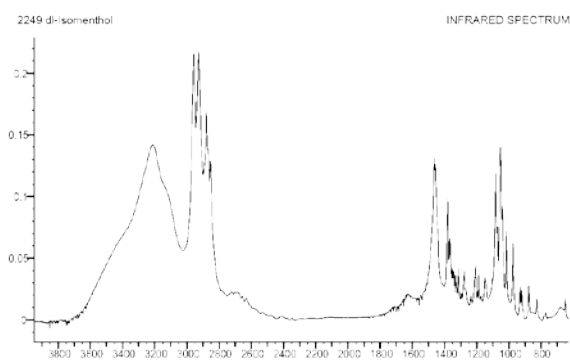
2247 MS



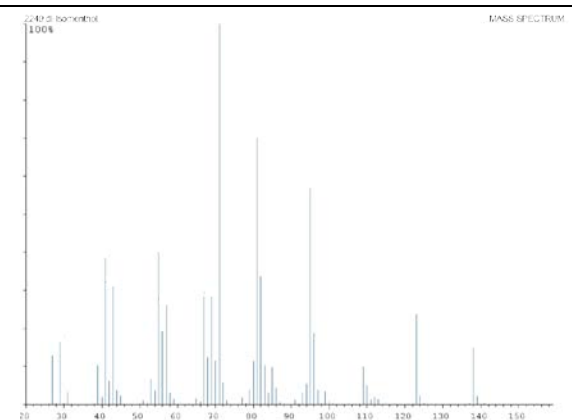
2248 MS



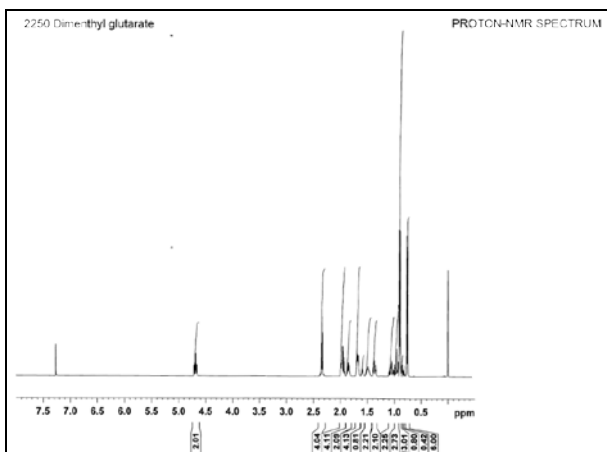
2249 HNMR



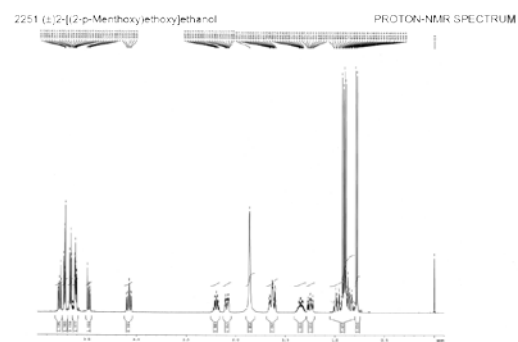
2249 IR



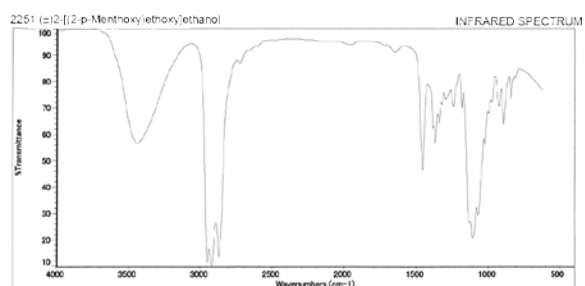
2249 MS



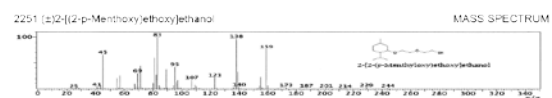
2250 HNMR



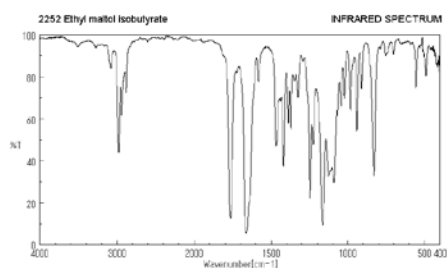
2251 HNMR



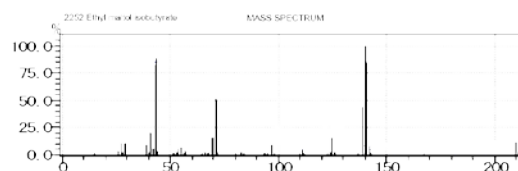
2251 IR



2251 MS



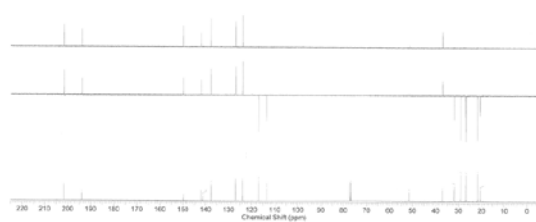
2252 IR



2252 MS

2253 Mixture of 1-Vinyl-3-cyclohexenecarbaldehyde and 4-Vinyl-1-cyclohexenecarbaldehyde

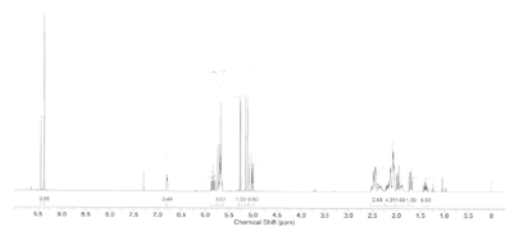
CARBON-13NMR SPECTRUM



2253 C13NMR

2253 Mixture of 1-Vinyl-3-cyclohexenecarbaldehyde and 4-Vinyl-1-cyclohexenecarbaldehyde

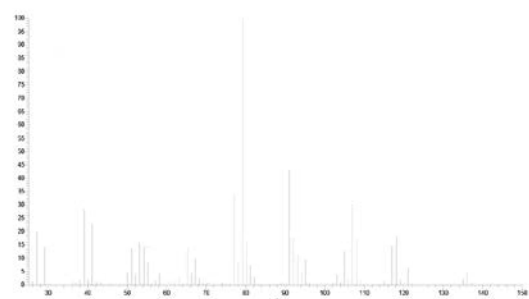
PROTON-NMR SPECTRUM



2253 HNMR

2253 1-Vinyl-3-cyclohexenecarbaldehyde

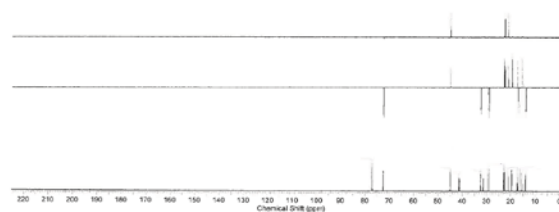
MASS SPECTRUM



2253 MS

2254 (1-Methyl-2-(1,2,2-trimethylbicyclo[3.1.0]hex-3-ylmethyl)cyclopropyl)methanol

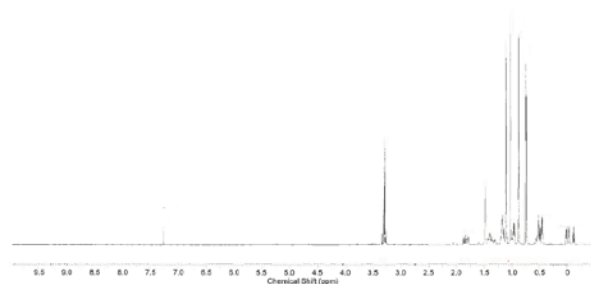
CARBON-13NMR SPECTRUM



2254 C13NMR

2254 (1-Methyl-2-(1,2,2-trimethylbicyclo[3.1.0]hex-3-ylmethyl)cyclopropyl)methanol

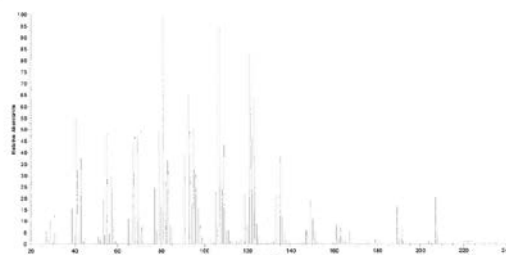
PROTON-NMR SPECTRUM



2254 HNMR

2254 (1-Methyl-2-(1,2,2-trimethylbicyclo[3.1.0]hex-3-ylmethyl)cyclopropyl)methanol

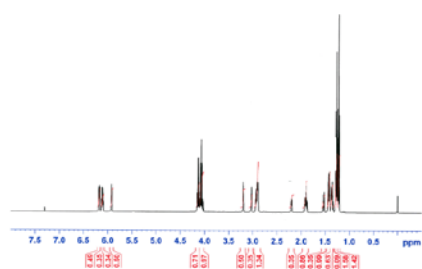
MASS SPECTRUM



2254 MS

2255 (±)-Bicyclo[2.2.1]hept-5-ene-2-carboxylic acid, ethyl ester

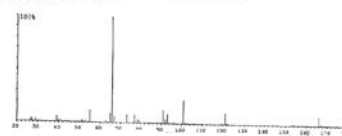
PROTON-NMR SPECTRUM



2255 HNMR

2255 (±)-Bicyclo[2.2.1]hept-5-ene-2-carboxylic acid, ethyl ester

MASS SPECTRUM

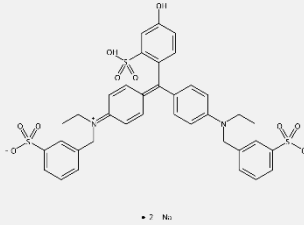


2255 MS

CORRIGENDUM

The following requests for corrections, reported to the JECFA secretariats, were evaluated by the 86th JECFA meeting and found to be necessary. These corrections, however, will only be made in the electronic versions and in the on-line database.

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 <u>mix</u> in 20 ml of arachid oil....	Correction
Curcumin (INS: 100(i)) 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
cis-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 L-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

Food additive	Original text	New text	Additional information
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) Monograph 20 (2017)	CAS No. 112696-00-8 (hydrated silica)	CAS No. 112926-00-8 (hydrated silica)	Transcription error
	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 the 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; No.: number
 Bolding and underlining for clarity only. This formatting will not be shown in the online database.

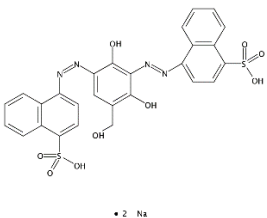
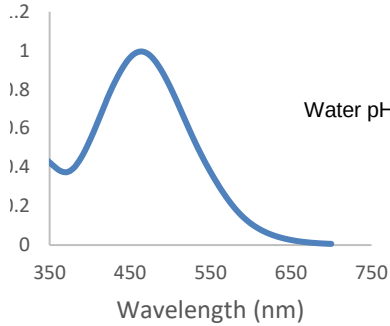
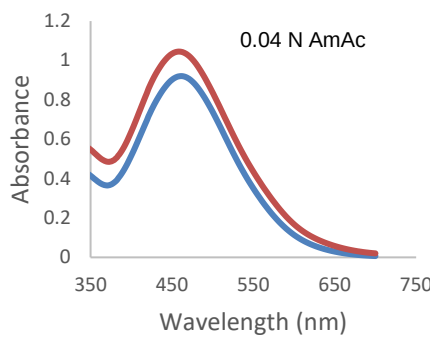
The criteria for several residual solvents are listed under the heading “Residual solvents” (see Fig. 1)

Figure1: 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:	
	Isopropanol:	Not more than 50 mg/kg
	Ethyl acetate:	

Table 1

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 $\lambda_{\max} = 464$ $A = 0.9957$ Spec abs = 403 $a = 40.3$ Water $\lambda_{\max} = 464$ $A = 0.9804$ Spec abs = 397 $a = 39.7$ 0.04 N AmAc $\lambda_{\max} = 461$ $A = 0.9206$ Spec abs = 373 $a = 37.3$	 Water pH 7
Brown HT Aluminium Lake	53.3 mg		Straight colour (blue) 0.04 N AmAc $\lambda_{\max} = 461$ $A = 0.9206$ Lake (red) 0.04 N AmAc $\lambda_{\max} = 458$ $A = 1.0451$	 0.04 N AmAc

ANNEX I: SUMMARY OF RECOMMENDATIONS FROM THE 86th JECFA



Food and Agriculture Organization
of the United Nations



World Health
Organization

JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES Eighty-sixth meeting Geneva, 12–21 June 2018

SUMMARY AND CONCLUSIONS *Issued 3 July 2018*

A meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) was held in Geneva, Switzerland, from 12 to 21 June 2018. The purpose of the meeting was to evaluate certain food additives (including flavouring agents).

Dr A. Mattia, Center for Food Safety and Applied Nutrition, United States Food and Drug Administration, served as Chairperson, and Dr. Richard Cantrill, Canada, served as Vice-Chairperson.

Dr M. Lipp, Office for Food Safety, Food and Agriculture Organization of the United Nations, and Dr A. Tritscher, Department of Food Safety and Zoonoses, World Health Organization, served as Joint Secretaries.

The present meeting was the eighty-sixth in a series of similar meetings. The tasks before the Committee were (a) to undertake safety evaluations of certain food additives (including flavouring agents); and (b) to review and prepare specifications for certain food additives (including flavouring agents).

The Committee evaluated the safety of eight food additives, revised the specifications for 19 other food additives (including 16 modified starches), evaluated 69 flavouring agents according to the revised Procedure for the Safety Evaluation of Flavouring Agents and revised the specifications for three flavouring agents.

The report of the meeting will be published in the WHO Technical Report Series. Its presentation will be similar to that of previous reports – namely, general considerations, comments on specific substances and recommendations for future work. An annex will include detailed tables (similar to the tables in this report) summarizing the main conclusions of the Committee in terms of acceptable daily intakes and other toxicological, dietary exposure and safety recommendations. Information on the specifications for the identity and purity of certain food additives (including flavouring agents) examined by the Committee will also be included.

The participants in the meeting are listed in Annex 1. Items of a general nature that the Committee would like to disseminate quickly are included in Annex 2. Future work and recommendations are listed in Annex 3.

Toxicological and dietary exposure monographs on most of the substances that were considered will be published in WHO Food Additives Series No. 77. New and revised specifications for the identity and purity of the compounds will be published in FAO JECFA Monographs 22.

More information on the work of JECFA is available at:

<http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/en/>

and

http://www.who.int/foodsafety/areas_work/chemical-risks/jecfa/en/

The issuance of this document does not constitute formal publication. The document may, however, be freely reviewed, abstracted, reproduced or translated, in whole or in part, but not for sale or use in conjunction with commercial purposes.

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 NOAELs for BMC ranged from 750-2000 mg/kg bw per day which were the highest doses tested. The Committee evaluated exposure to BMC for the copolymer and its monomers (n-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 (95 th 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 health concern.

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions
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. 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). 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 <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 454–2000 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

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions
Sorbitol syrup	-	give rise to concerns when taking into account the low dietary exposures. 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. 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	<u>R^a</u>
<u>Citric and fatty acid esters of glycerol</u>	<u>R, T^b</u>
<u>Glycerol ester of wood rosin</u>	<u>R^c</u>
<u>Modified starches</u>	<u>R^d, T</u>

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 recommended 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 that:

- 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 revised 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

N: new specifications

M: existing specifications maintained;

B. Carvone and structurally related substances

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class I			
Pinocarvyl 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

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
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
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-(2 <i>H</i>)-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>dl</i> -Isomenthol	2249	N	No safety concern
Dimethyl glutarate	2250	N	No safety concern
Menthol	427	M	No safety concern ^a
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
S)-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

Flavouring agents 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

^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 2. GENERAL INFORMATION

This section is deliberately blank

ANNEX 3. 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. Further studies to clarify the *in vivo* carcinogenic potential are required.

Citric and fatty acid esters of glycerol

The specifications of CITREM were made tentative, requiring 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 there was no data submitted for a suitable method of assay. Tentative specifications for NMC were prepared and made tentative requiring 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).
- 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 requests suitable microbiological acceptance criteria and supporting data for all modified starches.

Table 1. The annexes and the modified starches to which they apply along with required information:

ANNEX	Modification	Starches to which it applies	Information required
1	Minor fragmentation	INS 1400: Dextrin roasted starch; INS 1401: Acid treated starch; INS 1402: Alkaline treated starch; INS 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 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 1410: Monostarch phosphate; INS 1412: Distarch phosphate; INS 1413: Phosphated distarch phosphate; INS 1414: Acetylated distarch phosphate; INS 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 1420: Starch acetate; INS 1414: Acetylated distarch phosphate; INS 1422: Acetylated distarch adipate; INS 1451: Acetylated oxidized starch	Currently no additional information required.
5	Oxidation	INS 1404: Oxidized starch; INS 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 1450: Starch sodium octenyl succinate	Currently no additional information required.
7	Etherification with propylene epoxide	INS 1440: Hydroxypropyl starch; INS 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 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

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 three 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.

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.

COMPENDIUM OF FOOD ADDITIVE SPECIFICATIONS

Joint FAO/WHO Expert Committee on Food Additives
86th Meeting 2018

This document contains food additive specification monographs, analytical methods, and other information prepared at the eighty-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which was held in Geneva, 12–21 June 2018. The specification monographs provide information on the identity and purity of food additives used directly in foods or in food production. The main three objectives of these specifications are to identify the food additive that has been subjected to testing for safety, to ensure that the additives are of the quality required for use in food or in processing and to reflect and encourage good manufacturing practice. This publication and other documents produced by JECFA contain information that is useful to all those who work with or are interested in food additives and their safe use in food.

ISBN 978-92-5-131100-4 ISSN 1817-7077



9 7 8 9 2 5 1 3 1 1 0 0 4

CA2330EN/1/11.18

Evaluation of certain food additives

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



Food and Agriculture
Organization of the
United Nations



World Health
Organization



World Health
Organization

The World Health Organization (WHO) 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 Member States 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.

The WHO Technical Report Series makes available the findings of various international groups of experts that provide WHO with the latest scientific and technical advice on a broad range of medical and public health subjects. Members of such expert groups serve without remuneration in their personal capacities rather than as representatives of governments or other bodies; their views do not necessarily reflect the decisions or the stated policy of WHO.

To purchase WHO publications, please contact: WHO Press, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland (tel. +41 22 791 3264; fax: +41 22 791 4857; email: bookorders@who.int; <http://www.who.int/bookorders>).

Evaluation of certain food additives

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

*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*



Food and Agriculture
Organization of the
United Nations



World Health
Organization

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

(WHO technical report series; no. 1014)

ISBN 978-92-4-121023-2

ISSN 0512-3054

© World Health Organization and Food and Agriculture Organization of the United Nations, 2019

Some rights reserved. This work is available under the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 IGO licence (CC BY-NC-SA 3.0 IGO; <https://creativecommons.org/licenses/by-nc-sa/3.0/igo/>).

Under the terms of this licence, you may copy, redistribute and adapt the work for non-commercial purposes, provided the work is appropriately cited, as indicated below. In any use of this work, there should be no suggestion that the World Health Organization (WHO) or the Food and Agriculture Organization of the United Nations (FAO) endorse any specific organization, products or services. The use of the WHO or FAO logo is not permitted. If you adapt the work, then you must license your work under the same or equivalent Creative Commons licence. If you create a translation of this work, you should add the following disclaimer along with the suggested citation: "This translation was not created by the World Health Organization (WHO) or the Food and Agriculture Organization of the United Nations (FAO). WHO and FAO are not responsible for the content or accuracy of this translation. The original English edition shall be the binding and authentic edition".

Any mediation relating to disputes arising under the licence shall be conducted in accordance with the mediation rules of the World Intellectual Property Organization <http://www.wipo.int/amc/en/mediation/rules>.

Suggested citation. Evaluation of certain food additives: eighty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva: World Health Organization and Food and Agriculture Organization of the United Nations; 2019 (WHO technical report series; no. 1014). Licence: CC BY-NC-SA 3.0 IGO.

Cataloguing-in-Publication (CIP) data. CIP data are available at <http://apps.who.int/iris>.

Sales, rights and licensing. To purchase WHO publications, see <http://apps.who.int/bookorders>. To submit requests for commercial use and queries on rights and licensing, see <http://www.who.int/about/licensing>.

Third-party materials. If you wish to reuse material from this work that is attributed to a third party, such as tables, figures or images, it is your responsibility to determine whether permission is needed for that reuse and to obtain permission from the copyright holder. The risk of claims resulting from infringement of any third-party-owned component in the work rests solely with the user.

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of WHO or FAO concerning the legal or development status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

The mention of specific companies or of certain manufacturers' products, whether or not these have been patented, does not imply that they are endorsed or recommended by WHO or FAO in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

All reasonable precautions have been taken by WHO and FAO to verify the information contained in this publication. However, the published material is being distributed without warranty of any kind, either expressed or implied. The responsibility for the interpretation and use of the material lies with the reader. In no event shall WHO and FAO be liable for damages arising from its use.

This publication contains the collective views of an international group of experts and does not necessarily represent the decisions or the policies of WHO or FAO.

Design: Rania Spatha (<http://www.raniaspatha.com>)

Printed in Malta.

Contents

List of participants	v
List of abbreviations	viii
1. Introduction	1
1.1 Declarations of interests	1
1.2 Modification of the agenda	1
2. General considerations	3
2.1 Report from the Fiftieth Session of the Codex Committee on Food Additives (CCFA)	3
2.2 Update on activities relevant to JECFA	4
2.2.1 Update of <i>Environmental Health Criteria 240: Principles and methods for the risk assessment of chemicals in food (EHC 240)</i>	4
2.3 Food additive specifications and analytical methods	4
2.3.1 Corrigenda for specifications monographs	4
3. Specific food additives (other than flavouring agents)	9
3.1 Toxicological evaluation, exposure assessment and establishment of specifications	9
3.1.1 Anionic methacrylate copolymer	9
3.1.2 Basic methacrylate copolymer	17
3.1.3 Erythrosine	27
3.1.4 Indigotine	34
3.1.5 Lutein and lutein esters from <i>Tagetes erecta</i> and zeaxanthin (synthetic)	39
3.1.6 Neutral methacrylate copolymer	46
3.1.7 Sorbitol syrup	54
3.1.8 Spirulina extract	55
3.2 Revision of specifications and analytical methods	63
3.2.1 Cassia gum	63
3.2.2 Citric and fatty acid esters of glycerol	64
3.2.3 Glycerol ester of wood rosin	64
3.2.4 Modified starches	65
4. Flavouring agents	71
4.1 Toxicological evaluation and exposure assessment	71
4.1.1 Alicyclic primary alcohols, aldehydes, acids and related esters	71
4.1.2 Carvone and structurally related substances	78
4.1.3 Furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers	84
4.1.4 Linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters	98
4.1.5 Maltol and related substances	102
4.1.6 Menthol and structurally related substances	107
4.1.7 Miscellaneous nitrogen-containing substances	115
4.1.8 Saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids	119
4.2 Specifications of identity and purity of flavouring agents	124
4.2.1 New and maintained specifications	124

4.2.2 Revised specifications	125
5. Future work and recommendations	127
Acknowledgements	129
Annex 1	
Reports and other documents resulting from previous meetings of the Joint FAO/WHO Expert Committee on Food Additives	131
Annex 2	
Toxicological information and information on specifications	145
Annex 3	
Summary of the safety evaluation of the secondary components for flavouring agents with minimum assay values of less than 95%	153
Annex 4	
Meeting agenda	155

List of participants

Eighty-sixth meeting of the Joint FAO/WHO Expert Committee on
Food Additives
Geneva, 12–21 June 2018

Members

- Dr S. Barlow, Brighton, East Sussex, England, United Kingdom
- Dr J. Bend, Department of Pathology and Laboratory Medicine, Schulich Medicine & Dentistry, Western University, London, Ontario, Canada
- Dr D. Benford, Cheddington, London, England, United Kingdom
- Dr R. Cantrill, Halifax, Nova Scotia, Canada (*Vice-Chairperson*)
- Dr D. Folmer, Office of Food Additive Safety, Center for Food Safety and Applied Nutrition, United States Food and Drug Administration, College Park, Maryland, United States of America (USA) (*Joint Rapporteur*)
- Dr A. Mattia, Office of Food Additive Safety, Center for Food Safety and Applied Nutrition, United States Food and Drug Administration, College Park, Maryland, USA (*Chairperson*)
- Dr U. Mueller, Australian Pesticides and Veterinary Medicines Authority (APVMA), Symonston, Australian Capital Territory (ACT), Australia (*Joint Rapporteur*)
- Dr O.E. Orisakwe, University of Port Harcourt, Choba, Port Harcourt, Rivers State, Nigeria
- Dr J. Schlatter, Zurich, Switzerland
- Dr J. Smith, Bio|Food|Tech, Charlottetown, Prince Edward Island, Canada
- Dr M. Veerabhadra Rao, Hyderabad, India

Secretariat

- Ms Annamaria Bruno, Joint FAO/WHO Food Standards Programme, Food and Agriculture
Dr J.H. Andersen, National Food Institute, Technical University of Denmark, Lyngby, Denmark (*WHO Temporary Adviser*)
- Dr J.N. Barrows, Office of Cosmetics and Colors, Center for Food Safety and Applied Nutrition, United States Food and Drug Administration, College Park, Maryland, USA (*FAO Expert*)
- Dr C. Béchaux, ANSES, Maisons-Alfort, France (*WHO Temporary Adviser*)

- Dr M. DiNovi, Office of Food Additive Safety, Center for Food Safety and Applied Nutrition, United States Food and Drug Administration, College Park, Maryland, USA (*WHO Temporary Adviser*)¹
- Dr B. Fallico, Food Science and Technology Unit, University of Catania, Catania, Italy (*FAO Expert*)
- Mr Y. Fan, China National Center for Food Safety Risk Assessment, Beijing, China (*CCFA Chairperson*)
- Dr M.J.F. Fernandez, Universidad Miguel Hernández, Alicante, Spain (*FAO Expert*)
- Dr B. Fields, Food Standards Australia New Zealand, Barton, Australian Capital Territory (ACT), Australia (*WHO Temporary Adviser*)
- Ms T. Hambridge, Food Standards Australia New Zealand (FSANZ), Kingston, ACT, Australia (*WHO Temporary Adviser*)
- Ms F. Hill, Food Standards Agency, London, United Kingdom (*WHO Temporary Adviser*)
- Dr S.M.F. Jeurissen, Centre for Nutrition, Prevention and Health Services, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands (*WHO Temporary Adviser*)
- Dr X. Jia, Laboratory of Toxicology, China National Center for Food Safety Risk Assessment, Beijing, China (*WHO Temporary Adviser*)
- Dr S. Kim, Department of Food Safety and Zoonoses, World Health Organization, Geneva, Switzerland (*WHO Secretariat*)
- Dr K. Laurvick, United States Pharmacopeial Convention, Rockville, Maryland, USA (*FAO Expert*)
- Dr M. Lipp, Agriculture and Consumer Protection Department, Food and Agriculture Organization of the United Nations, Rome, Italy (*FAO Joint Secretary*)
- Dr P. Mosesso, Department of Ecological and Biological Sciences, Università degli Studi della Tuscia, Viterbo, Italy (*WHO Temporary Adviser*)
- Ms J. Odrowaz, Toronto, Ontario, Canada (*WHO Technical Editor*)
- Dr K. Petersen, Department of Food Safety and Zoonoses, World Health Organization, Geneva, Switzerland (*WHO Secretariat*)
- Dr L. Rosenfeld, Division of Petition Review, Office of Food Additive Safety, Center for Food Safety and Applied Nutrition, United States Food and Drug Administration, College Park, Maryland, USA (*WHO Temporary Adviser*)
- Dr J. Rotstein, Pre-Market Toxicology Assessment Section, Chemical Health Hazard Assessment Division, Bureau Chemical Safety, Food Directorate, Health Products and Food Branch, Health Canada, Ottawa, Ontario, Canada (*WHO Temporary Adviser*)

- Dr J.R. Srinivasan, Division of Biotech and GRAS Notice Review, Office of Food Additive Safety, Center for Food Safety and Applied Nutrition, United States Food and Drug Administration, College Park, Maryland, USA (*FAO Expert*)¹
- Dr N. Sugimoto, Division of Food Additives, National Institute of Health Sciences (NIHS), Kanagawa, Japan
- Dr S. Takasu, Division of Pathology, Biological Safety Research Center, National Institute of Health Sciences, Kawasaki-shi, Kanagawa, Japan (*WHO Temporary Adviser*)
- Dr A. Tritscher, Department of Food Safety and Zoonoses, World Health Organization, Geneva, Switzerland (*WHO Joint Secretary*)
- Dr T. Umemura, Faculty of Animal Science Technology, Yamazaki Gakuen University, Tokyo, Japan (*WHO Temporary Adviser*)
- Dr X. Yang, Food Safety and Health Research Center, Southern Medical University, Guangzhou, Guangdong Province, China (*WHO Temporary Adviser*)
- Ms L. Zhang, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization of the United Nations, Rome, Italy (*Codex Secretariat*)

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

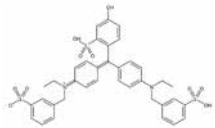
The following requests for corrections, reported to the JECFA Secretariat, were evaluated by the eighty-sixth Committee and found to be necessary (see [Table 1](#)). These corrections, however, will only be made in the online database for specifications.

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)

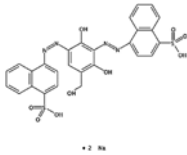
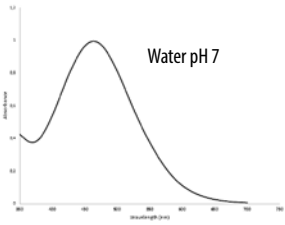
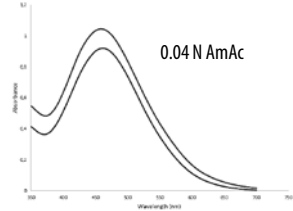
Food additive	Original text	New text	Additional information
Monograph 20 (2017)	Pyrogenic silica is produced in an essentially anhydrous state, whereas the wet process products are obtained as hydrates or contain surface absorbed water.	Pyrogenic silica is produced in an essentially anhydrous state,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

<u>Residual solvents</u> (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 (CIFOcOss) and data from Australia and New Zealand submitted to the Committee. A comprehensive literature search was also conducted; no additional studies were identified.

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

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

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

Evaluation

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

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

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

Table 3
Summary of range of estimated exposures to AMC, its monomers and total methacrylic acid from uses in food supplements for average and high exposures

Population group	Range of estimated dietary exposures ^{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.

References

1. EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS): Scientific Opinion of the safety of anionic methacrylate copolymer for the proposed uses as a food additive. *EFSA J.* 2010;8(7):1656 [25 pp.] doi:10.2903/j.efsa.2010.1656.
2. Report of the Forty-ninth Session of the Codex Committee on Food Additives, Macao SAR, China, 20–24 March 2017. Rome: Food and Agriculture Organization of the United Nations; and Geneva: World Health Organization, Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2017 (REP17/FA).
3. Absorption, distribution and excretion study in the rat following a 13-day administration period. Huntingdon Life Sciences, Eye, Suffolk, England, United Kingdom; 1999. Unpublished study report no. 010/984481 submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
4. Bratt H, Hathway DE. Fate of methyl methacrylate in rats. *Br J Cancer.* 1977;36(1):114–9 [cited in reference 16].
5. Canadian Environmental Protection Act: Priority Substances List assessment report: Methyl methacrylate. Prepared by Health Canada and Environment Canada. Ottawa: Government of Canada; 1993 (Cat. No.: En40-215/10E) [cited in reference 16].
6. Methyl methacrylate – CAS No. 80-62-6. Brussels: European Centre for Ecotoxicology and Toxicology of Chemicals; 1995 (Joint Assessment of Commodity Chemicals No. 30) [cited in reference 16].
7. Vodicka P, Gut I, Frantík E. Effects of inhaled acrylic acid derivatives in rats. *Toxicology.* 1990;65(1–2):209–21.
8. Sapota A. The disposition and metabolism of methyl acrylate in male Wistar albino rats. *Pol J Occup Med Environ Health.* 1993;6(2):185–93.
9. Bereznowski Z, Mariewski M, Somolenski RT. High performance liquid chromatographic determination of methacrylate in blood serum. *Biomed Chromatogr.* 1994;8(1):42–4 [cited in European Commission, 2002].
10. Acute oral toxicity study in the rat. Research Toxicology Centre, Rome, Italy; 1995. Unpublished study report no. 5090/T/211/95 submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
11. Four-week oral toxicity study in rats followed by a 2-week recovery period. Research Toxicology Centre, Rome, Italy; 1998. Unpublished study report no. 861/T/215/98 submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
12. Twenty-six week oral toxicity study in rats followed by a 4-week recovery period. Unpublished study by Research Toxicology Centre, Rome, Italy; 2000. Report no. 7250/T/346/2000 submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.

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

3.1.2 Basic methacrylate copolymer

Explanation

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

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

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

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

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

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

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

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

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

Chemical and technical considerations

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

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

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

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

Biochemical aspects

BMC

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

Residual monomers

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

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

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

Toxicological studies

BMC

In acute toxicity studies in mice, no effects were observed after oral, intraperitoneal and subcutaneous administration of BMC (purity >98%) at 3.0, 2.0 and 1.0 g/kg bw, respectively, although deaths occurred at higher doses via the intraperitoneal and oral routes. In rats, the oral median lethal dose (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.

References

1. EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS); Scientific Opinion on the use of basic methacrylate copolymer as a food additive. EFSA J. 2010;8(2):1513 [23 pp.] doi:10.2903/j.efsa.2010.1513.
2. Report of the Forty-ninth Session of the Codex Committee on Food Additives, Macao SAR, China, 20–24 March 2017. Rome: Food and Agriculture Organization of the United Nations; and Geneva: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2017 (REP17/FA).
3. [¹⁴C]-Eudragit-E-100: The excretion and tissue distribution of radioactivity after oral administration to rats. Report no. 79/RGC024/278. Life Science Research, Stock, Essex, England, United Kingdom; 1979. Unpublished study report. Submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.

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

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

3.1.3 Erythrosine

Explanation

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

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

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

At the thirtieth meeting the Committee reduced the temporary ADI to 0–0.6 mg/kg bw, based on biochemical effects of erythrosine on thyroid hormone metabolism and regulation in rats. The Committee at the thirtieth meeting requested further data from pharmacokinetic studies relating the amount of erythrosine absorbed to the amount ingested to enable the establishment of a correlation between blood/tissue erythrosine levels and effects on the thyroid. At the thirty-third meeting the Committee further reduced the temporary ADI to 0–0.05 mg/kg bw. This ADI was based on a NOAEL from a study showing slightly increased thyroid-stimulating hormone (TSH) responsiveness in humans ingesting erythrosine at 60 mg per person per day (equivalent to 1 mg/kg bw per day) for 14 days, and applying an uncertainty 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.

References

1. Report of the Forty-ninth Session of the Codex Committee on Food Additives, Macao SAR, China, 20–24 March 2017. Rome: Food and Agriculture Organization of the United Nations; and Geneva: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2017 (REP17/FA).
2. Obrist J, LeVan L, Puhl RJ, Duan RJ. Final report: Metabolism of FD&C Red No. 3 in rats, study no. 6145–100. Unpublished report by Hazleton Laboratories America, Inc., 1986. Submitted to WHO by the Certified Color Manufacturers Association, Inc., Washington, DC, USA.

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

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

3.1.4 Indigotine

Explanation

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

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

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

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

Chemical and technical considerations

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

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

References

1. Hansen WH, Fitzhugh OG, Nelson AA, Davis KJ. Chronic toxicity of two food colors, brilliant blue FCF and indigotine. *Toxicol Appl Pharmacol*. 1966;8(1):29–36.
2. Report of the Forty-ninth Session of the Codex Committee on Food Additives, Macao SAR, China, 20–24 March 2017. Rome: Food and Agriculture Organization of the United Nations and Geneva: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2017 (REP17/FA).
3. Lethco EJ, Webb JM. The fate of FD&C Blue No. 2 in rats. *J Pharmacol Exp Ther*. 1966;154(2):384–9.
4. Singh S, Das M, Khanna SK. Bio-metabolism of green S and indigo carmine through caecal microflora of rats. *Biochem Biophys Res Commun*. 1993;195(1):490–96.
5. Kuno N, Mizutani T. Influence of synthetic and natural food dyes on activities of CYP2A6, UGT1A6, and UGT2B7. *J Toxicol Environ Health A*. 2005;68(16):1431–44.
6. Mizutani T. Toxicity of xanthene food dyes by inhibition of human drug-metabolizing enzymes in a noncompetitive manner. *J Environ Public Health*. 2009;953952:1–9. doi:10.1155/2009/953952.
7. Gaunt IF, Kiss IS, Grasso P, Gangolli SD. Short-term toxicity study on indigo carmine in the pig. *Food Cosmet Toxicol*. 1969;7(1):17–24.
8. Hooson J, Gaunt IF, Kiss IS, Grasso P, Butterworth KR. Long-term toxicity of indigo carmine in mice. *Food Cosmet Toxicol*. 1975;13(2):167–76.
9. Borzelleca JF, Hogan GK. Chronic toxicity/carcinogenicity study of FD & C Blue No. 2 in mice. *Food Chem Toxicol*. 1985;23(8):719–22.

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

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

Explanation

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

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

Lutein esters contain lutein (all-*E*,3*R*,3'*R*,6'*R*)- 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.

References

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

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

3.1.6 Neutral methacrylate copolymer

Explanation

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

References

1. Polyacrylate Dispersion 30 Per Cent. 9 01/2017:0733. European Pharmacopeia; 2017.
2. United States Pharmacopeia and National Formulary (USP 41-NF 36); Ethyl acrylate and methyl methacrylate copolymer dispersion. Vol. 1. Rockville, Maryland: United States Pharmacopeial Convention; 2017.
3. Report of the Forty-ninth Session of the Codex Committee on Food Additives, Macao SAR, China, 20–24 March 2017. Rome: Food and Agriculture Organization of the United Nations and Geneva: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2017 (REP17/FA).
4. The excretion and tissue distribution of radioactivity after oral administration to rats. Huntingdon Life Sciences, Suffolk, England, United Kingdom; 1986. Report no. RTS 010/984481(2nd amendment). Unpublished study report submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
5. Bratt H, Hathway DE. Fate of methyl methacrylate in rats. *Br J Cancer*. 1977;36(1):114–9 [cited in reference 16].
6. Canadian Environmental Protection Act: Priority Substances List assessment report: Methyl methacrylate. Prepared by Health Canada and Environment Canada. Ottawa: Government of Canada; 1993 (Cat. No.: En40-215/10E) [cited in reference 16].
7. Methyl methacrylate – CAS No. 80-62-6. Brussels: European Centre for Ecotoxicology and Toxicology of Chemicals; 1995. (Joint Assessment of Commodity Chemicals No. 30) [cited in reference 16].
8. Screening assessment for the challenge 2-propenoic acid, ethyl ester (ethyl acrylate). Chemical Abstracts Service Registry Number 140-88-5. Ottawa (ON): Environment Canada, Health Canada; 2011.
9. The acute toxicity of preparations 2807B and 2837B on oral administration in rats. Unpublished study by Laboratory for Pharmacology and Toxicology, Hamburg-Hausbruch, Germany; 1971. Submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
10. The acute toxicity of preparations 2807B and 2837B on oral administration in dogs when administered in the feedstuff. Unpublished study by Laboratory for Pharmacology and Toxicology, Hamburg-Hausbruch, Germany; 1972a. Submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
11. Subacute toxicity study with rats by oral administration for one month and the recovery study. Unpublished study by BoZo Research Centre, Co Ltd, Japan; 1981. Submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.

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

3.1.7 Sorbitol syrup

Explanation

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

Evaluation

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

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

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

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

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

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

No toxicological monograph was prepared.

Reference

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

3.1.8 *Spirulina* extract

Explanation

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

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

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

Chemical and technical considerations

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

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

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

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

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

Biochemical aspects

ADME data on spirulina extract are not available.

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

An in vitro simulated gastric fluid digestion assay demonstrated that the protein portion of the C-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). F_1 and F_2 offspring were not directly exposed to the test material. At 30% dried spirulina in the diet, reduced F_1 pup weight at birth and reduced F_1 survival rate on postnatal days 0–4 were reported; no effects were observed in the F_2 animals [26]. The Committee noted that there was no effect on pup survival rate in a 2-generation toxicity study in which F_0 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.

References

1. Report of the Forty-ninth Session of the Codex Committee on Food Additives, Macao SAR, China, 20–24 March 2017. Rome: Food and Agriculture Organization of the United Nations; and Geneva: World Health Organization. Rome: Food and Agriculture Organization of the United Nations and Geneva: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2017 (REP17/FA).

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

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

3.2 Revision of specifications and analytical methods

3.2.1 Cassia gum

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

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

3.2.2 Citric and fatty acid esters of glycerol

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

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

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

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

A Chemical and Technical Assessment was prepared.

3.2.3 Glycerol ester of wood rosin

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

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

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

References

1. Report of the Forty-ninth Session of the Codex Committee on Food Additives, Macao SAR, China, 20–24 March 2017. Rome: Food and Agriculture Organization of the United Nations and Geneva: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2017 (REP17/FA).

3.2.4 Modified starches

General considerations

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

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

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

The Committee noted the following:

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

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

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

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

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

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

Table 10

List of modified starches considered and applicable annexes

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

INS: International Numbering System for Food Additives

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

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

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

Technical considerations

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

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

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

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

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

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

Table 11
Information required in the annexes

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

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

References

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

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

4. Flavouring agents

4.1 Toxicological evaluation and exposure assessment

4.1.1 Alicyclic primary alcohols, aldehydes, acids and related esters

Introduction

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

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

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

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

Two of the six previously evaluated flavouring agents, *p*-mentha-1,8-dien-7-al (No. 973) and formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene (No. 980), contain an 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}

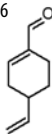
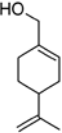
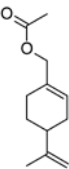
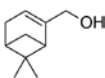
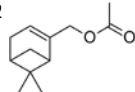
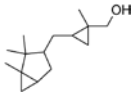
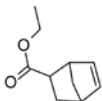
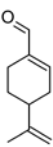
Flavouring agent	No.	CAS no. and structure	Step 4 ^e Does the highest dietary exposure estimate exceed the threshold of toxicological concern?	Conclusion based on current estimated dietary exposure
Structural class I				
Mixture of 1-vinyl-3-cyclohexenecarbaldehyde and 4-vinyl-1-cyclohexenecarbaldehyde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

References

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

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

4.1.2 Carvone and structurally related substances

Introduction

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

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

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

Carvone (Nos 380.1 and 380.2) was evaluated at the eleventh meeting ([Annex 1](#), reference 14) at which a 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.

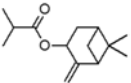
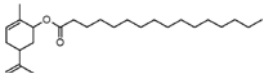
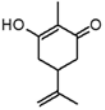
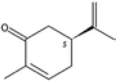
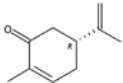
[Table 13](#) summarizes the evaluations of the three flavouring agents (Nos 2242–2244) in the group of carvone and structurally related substances.

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				
Pinocaryyl 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.

References

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

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

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

Introduction

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

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

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

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

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

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

Assessment of dietary exposure

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

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

Absorption, distribution, metabolism and excretion

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

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

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

Consideration of genotoxicity data

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

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

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

Application of the Procedure for the Safety Evaluation of Flavouring Agents

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Consideration of combined intakes from use as flavouring agents

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

Consideration of secondary components

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

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

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

Conclusions

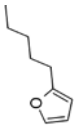
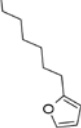
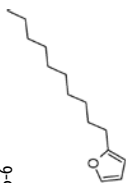
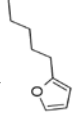
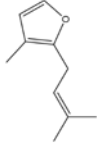
The Committee concluded that the 39 previously evaluated flavouring agents in this group of furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers that were re-evaluated at the present meeting (Nos 1491–1529 and 2103–2105)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

An addendum to the monograph was prepared.

References

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

Table 14
Summary of the results of the safety evaluations of furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers used as flavouring agents^{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

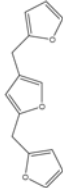
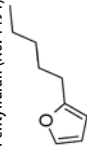
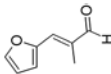
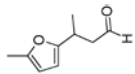
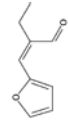
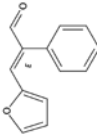
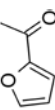
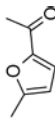
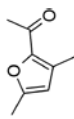
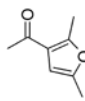
Step 4 Does the higher of the two predicted dietary exposure estimates exceed the threshold of toxicological concern value for the structural class?*			Step 5 Does a NOAEL exist for the flavouring agent or a structural relative that provides an adequate margin of exposure?		Structural relative name (No.)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

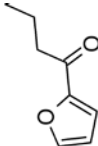
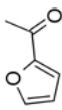
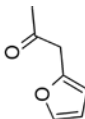
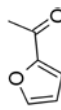
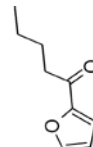
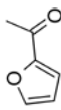
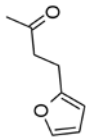
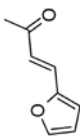
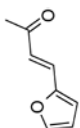
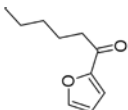
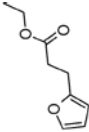
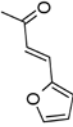
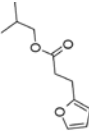
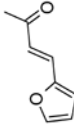
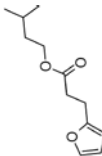
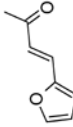
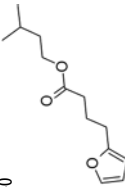
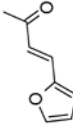
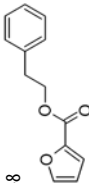
Flavouring agent	No.	CAS no. and structure	Step 4		Step 5 Does a NOAEL exist for the flavouring agent or a structural relative that provides an adequate margin of exposure?	Structural relative name (No.) and Structure	Conclusion based on current estimated dietary exposure
			Does the higher of the two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

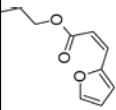
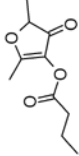
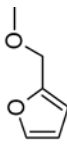
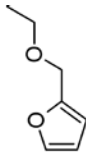
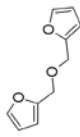
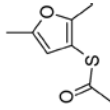
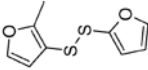
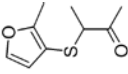
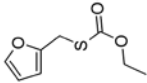
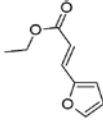
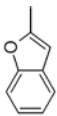
Step 4		Step 5		Conclusion based on current estimated dietary exposure
Flavouring agent	No.	Does the higher of the two predicted dietary exposure estimates exceed the threshold of toxicological concern value for the structural class?	Does a NOAEL exist for the flavouring agent or a structural relative 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.

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

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

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

Introduction

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

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

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

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

Assessment of dietary exposure

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

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

Absorption, distribution, metabolism and excretion

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

The aliphatic esters are expected to hydrolyse to their analogous unsaturated aliphatic alcohol and carboxylic acids during passage through the gastrointestinal tract [4, 5, 6]. The resulting linear and branched-chain unsaturated primary alcohols are expected to be absorbed and further oxidized to their analogous aldehydes and acids, and rapidly absorbed [7, 8]. The absorbed aldehydes are oxidized to their analogous unsaturated carboxylic acids. These unsaturated carboxylic acids undergo further enzymatic conversion prior to entry into the 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.

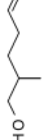
No adequate studies on the additional flavouring agents were available. For previously evaluated flavouring agents in this group, studies of acute toxicity (Nos 336, 1269, 1286 and 1640), studies of short-term toxicity (Nos 330, 332 and 333) and studies of genotoxicity (Nos 315, 329, 330, 333, 334, 346,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

References

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

4.1.5 Maltol and related substances

Introduction

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

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

Maltol (No. 1480) was evaluated at the eleventh meeting ([Annex 1](#), reference 14), when a temporary ADI of 0–1 mg/kg bw was established because no long-term studies of toxicity were available. At the eighteenth meeting ([Annex 1](#), reference 35), the Committee withdrew the temporary ADI because the results of long-term studies of toxicity requested at the previous 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.

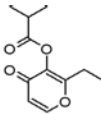
Step 4. The highest estimated dietary exposure for maltol (No. 1480) is above the threshold of toxicological concern (i.e. 540 $\mu\text{g/day}$ for class II). Accordingly, the evaluation of this flavouring agent proceeded to Step 5 of the Procedure.

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.

References

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

4.1.6 Menthol and structurally related substances

Introduction

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

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

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

Menthol (No. 427) was first evaluated by the Committee at the eleventh meeting when an unconditional ADI of 0–0.2 mg/kg bw and a 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

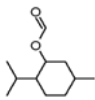
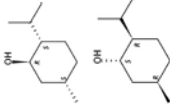
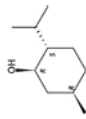
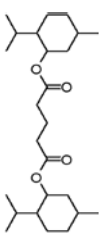
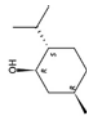
For eight previously evaluated flavouring agents in this group, additional studies of metabolism (No. 429); studies of acute toxicity (Nos 430, 1854 and 1856); and studies of genotoxicity (Nos 429, 430, 431, 445, 1856 and 1857) were available.

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.

References

1. Principles and methods for the risk assessment of chemicals in food. A joint publication of the Food and Agriculture Organization of the United Nations and the World Health Organization. Geneva: World Health Organization, International Programme on Chemical Safety; 2009 (Environmental Health Criteria 240).
2. Nijssen LM, van Ingen-Visscher CA, Donders JJH. Volatile compounds in food, version 16.4 [database]. Zeist, the Netherlands: TNO Triskelion B.V.; 2017 (<http://www.vcf-online.nl/VcfHome.cfm>, accessed 15 November 2017).
3. Report of the Forty-eighth Session of the Codex Committee On Food Additives. Xi'an, China, 14–18 March 2016. Rome: Food and Agriculture Organization of the United Nations; and Geneva: World; 2016 (REP16/FA).
4. 2015 IOFI Global Poundage Survey. International Organization of the Flavor Industry, Brussels; 2017a. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
5. Interim inquiry on volume use and added use levels for flavoring agents to be presented at the JECFA 86th meeting. Private communication to the International Organization of the Flavor Industry, Brussels; 2017b. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
6. Heymann E. Carboxylesterases and amidases. In: Jakoby WB, editor, Enzymatic basis of detoxification. New York, NY, USA: Academic Press; 1980.
7. Anders MW. Biotransformation and bioactivation of xenobiotics by the kidney. In: Hutson DH, Caldwell J, Paulson GD, editors. Intermediary xenobiotic metabolism in animals. New York, NY, USA: Taylor and Francis; 1989.

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

4.1.7 Miscellaneous nitrogen-containing substances

Introduction

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

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

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

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

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

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

Assessment of dietary exposure

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

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

Absorption, distribution, metabolism and excretion

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

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

Application of the Procedure for the Safety Evaluation of Flavouring Agents

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

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

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

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

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

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

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

Consideration of combined intakes from use as flavouring agents

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

Consideration of additional data on previously evaluated flavouring agents

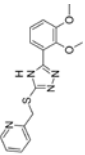
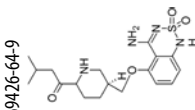
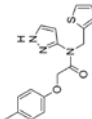
For previously evaluated flavouring agents in this group, studies of acute toxicity (No. 1566 and 1889), genotoxicity (No. 1566) and reproductive/developmental toxicity (No 2161) were available.

Conclusions

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

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

Table 18
Summary of the results of the safety evaluations of miscellaneous nitrogen-containing substances^{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
(S)-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

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

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

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

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

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

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

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

Assessment of dietary exposure

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

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

Absorption, distribution, metabolism and excretion

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

The substances in this group share common metabolic pathways, and are expected to be absorbed into the gastrointestinal tract [4, 5]. Shorter branched-chain aliphatic alcohols, aldehydes and acids undergo β -oxidation cleavage, with intermediates metabolized to 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

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

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

4.2 Specifications of identity and purity of flavouring agents

4.2.1 New and maintained specifications

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

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

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

4.2.2 Revised specifications

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

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

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

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



5. Future work and recommendations

Specific food additives (other than flavouring agents)

Anionic methacrylate copolymer

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

Citric and fatty acid esters of glycerol

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

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

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

Neutral methacrylate copolymer

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

Spirulina extract

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

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

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

Modified starches

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

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

Flavouring agents

Carvone and structurally related substances

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

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

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

Maltol and related substances

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

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

The ADI for ethyl maltol was maintained.

Acknowledgements

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.
47. Evaluation of certain food additives and contaminants (Twenty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 631, 1978.
48. Summary of toxicological data of certain food additives and contaminants. WHO Food Additives Series, No. 13, 1978.
49. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 7, 1978.
50. Evaluation of certain food additives (Twenty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 648, 1980, and corrigenda.
51. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 14, 1980.
52. Specifications for identity and purity of food colours, flavouring agents, and other food additives. FAO Food and Nutrition Paper, No. 12, 1979.
53. Evaluation of certain food additives (Twenty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 653, 1980.
54. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 15, 1980.
55. Specifications for identity and purity of food additives (sweetening agents, emulsifying agents, and other food additives). FAO Food and Nutrition Paper, No. 17, 1980.
56. Evaluation of certain food additives (Twenty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 669, 1981.
57. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 16, 1981.
58. Specifications for identity and purity of food additives (carrier solvents, emulsifiers and stabilizers, enzyme preparations, flavouring agents, food colours, sweetening agents, and other food additives). FAO Food and Nutrition Paper, No. 19, 1981.
59. Evaluation of certain food additives and contaminants (Twenty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 683, 1982.
60. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 17, 1982.
61. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 25, 1982.

62. Evaluation of certain food additives and contaminants (Twenty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 696, 1983, and corrigenda.
63. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 18, 1983.
64. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 28, 1983.
65. Guide to specifications – General notices, general methods, identification tests, test solutions, and other reference materials. FAO Food and Nutrition Paper, No. 5, Rev. 1, 1983.
66. Evaluation of certain food additives and contaminants (Twenty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 710, 1984, and corrigendum.
67. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 19, 1984.
68. Specifications for the identity and purity of food colours. FAO Food and Nutrition Paper, No. 31/1, 1984.
69. Specifications for the identity and purity of food additives. FAO Food and Nutrition Paper, No. 31/2, 1984.
70. Evaluation of certain food additives and contaminants (Twenty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 733, 1986, and corrigendum.
71. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 34, 1986.
72. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 20. Cambridge University Press, 1987.
73. Evaluation of certain food additives and contaminants (Thirtieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 751, 1987.
74. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 21. Cambridge University Press, 1987.
75. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 37, 1986.
76. Principles for the safety assessment of food additives and contaminants in food. WHO Environmental Health Criteria, No. 70. Geneva, World Health Organization, 1987 (out of print). The full text is available electronically at www.who.int/pes.
77. Evaluation of certain food additives and contaminants (Thirty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 759, 1987, and corrigendum.
78. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 22. Cambridge University Press, 1988.
79. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 38, 1988.
80. Evaluation of certain veterinary drug residues in food (Thirty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 763, 1988.

81. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 23. Cambridge University Press, 1988.
82. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41, 1988.
83. Evaluation of certain food additives and contaminants (Thirty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 776, 1989.
84. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 24. Cambridge University Press, 1989.
85. Evaluation of certain veterinary drug residues in food (Thirty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 788, 1989.
86. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 25, 1990.
87. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/2, 1990.
88. Evaluation of certain food additives and contaminants (Thirty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 789, 1990, and corrigenda.
89. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 26, 1990.
90. Specifications for identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 49, 1990.
91. Evaluation of certain veterinary drug residues in food (Thirty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 799, 1990.
92. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 27, 1991.
93. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/3, 1991.
94. Evaluation of certain food additives and contaminants (Thirty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 806, 1991, and corrigenda.
95. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 28, 1991.
96. Compendium of food additive specifications (Joint FAO/WHO Expert Committee on Food Additives (JECFA)). Combined specifications from 1st through the 37th meetings, 1956–1990. Rome, Food and Agriculture Organization of the United Nations, 1992 (2 volumes).
97. Evaluation of certain veterinary drug residues in food (Thirty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 815, 1991.
98. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 29, 1991.
99. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/4, 1991.
100. Guide to specifications – General notices, general analytical techniques, identification tests, test solutions, and other reference materials. FAO Food and Nutrition Paper, No. 5, Rev. 2, 1991.
101. Evaluation of certain food additives and naturally occurring toxicants (Thirty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 828, 1992.

102. Toxicological evaluation of certain food additives and naturally occurring toxicants. WHO Food Additives Series, No. 30, 1993.
103. Compendium of food additive specifications: addendum 1. FAO Food and Nutrition Paper, No. 52, 1992.
104. Evaluation of certain veterinary drug residues in food (Fortieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 832, 1993.
105. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 31, 1993.
106. Residues of some veterinary drugs in animals and food. FAO Food and Nutrition Paper, No. 41/5, 1993.
107. Evaluation of certain food additives and contaminants (Forty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 837, 1993.
108. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 32, 1993.
109. Compendium of food additive specifications: addendum 2. FAO Food and Nutrition Paper, No. 52, Add. 2, 1993.
110. Evaluation of certain veterinary drug residues in food (Forty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 851, 1995.
111. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 33, 1994.
112. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/6, 1994.
113. Evaluation of certain veterinary drug residues in food (Forty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 855, 1995, and corrigendum.
114. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 34, 1995.
115. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/7, 1995.
116. Evaluation of certain food additives and contaminants (Forty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 859, 1995.
117. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 35, 1996.
118. Compendium of food additive specifications: addendum 3. FAO Food and Nutrition Paper, No. 52, Add. 3, 1995.
119. Evaluation of certain veterinary drug residues in food (Forty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 864, 1996.
120. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 36, 1996.
121. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/8, 1996.
122. Evaluation of certain food additives and contaminants (Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 868, 1997.
123. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 37, 1996.

124. Compendium of food additive specifications, addendum 4. FAO Food and Nutrition Paper, No. 52, Add. 4, 1996.
125. Evaluation of certain veterinary drug residues in food (Forty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 876, 1998.
126. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 38, 1996.
127. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/9, 1997.
128. Evaluation of certain veterinary drug residues in food (Forty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 879, 1998.
129. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 39, 1997.
130. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/10, 1998.
131. Evaluation of certain food additives and contaminants (Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 884, 1999.
132. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 40, 1998.
133. Compendium of food additive specifications: addendum 5. FAO Food and Nutrition Paper, No. 52, Add. 5, 1997.
134. Evaluation of certain veterinary drug residues in food (Fiftieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 888, 1999.
135. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 41, 1998.
136. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/11, 1999.
137. Evaluation of certain food additives (Fifty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 891, 2000.
138. Safety evaluation of certain food additives. WHO Food Additives Series, No. 42, 1999.
139. Compendium of food additive specifications, addendum 6. FAO Food and Nutrition Paper, No. 52, Add. 6, 1998.
140. Evaluation of certain veterinary drug residues in food (Fifty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 893, 2000.
141. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 43, 2000.
142. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/12, 2000.
143. Evaluation of certain food additives and contaminants (Fifty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 896, 2000.
144. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 44, 2000.

145. Compendium of food additive specifications, addendum 7. FAO Food and Nutrition Paper, No. 52, Add. 7, 1999.
146. Evaluation of certain veterinary drug residues in food (Fifty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 900, 2001.
147. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 45, 2000.
148. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/13, 2000.
149. Evaluation of certain food additives and contaminants (Fifty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 901, 2001.
150. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 46, 2001.
151. Compendium of food additive specifications: addendum 8. FAO Food and Nutrition Paper, No. 52, Add. 8, 2000.
152. Evaluation of certain mycotoxins in food (Fifty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 906, 2002.
153. Safety evaluation of certain mycotoxins in food. WHO Food Additives Series, No. 47/FAO Food and Nutrition Paper, No. 74, 2001.
154. Evaluation of certain food additives and contaminants (Fifty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 909, 2002.
155. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 48, 2002.
156. Compendium of food additive specifications: addendum 9. FAO Food and Nutrition Paper, No. 52, Add. 9, 2001.
157. Evaluation of certain veterinary drug residues in food (Fifty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 911, 2002.
158. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 49, 2002.
159. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/14, 2002.
160. Evaluation of certain food additives and contaminants (Fifty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 913, 2002.
161. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 50, 2003.
162. Compendium of food additive specifications: addendum 10. FAO Food and Nutrition Paper, No. 52, Add. 10, 2002.
163. Evaluation of certain veterinary drug residues in food (Sixtieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 918, 2003.
164. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 51, 2003.
165. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/15, 2003.

166. Evaluation of certain food additives and contaminants (Sixty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 922, 2004.
167. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 52, 2004.
168. Compendium of food additive specifications: addendum 11. FAO Food and Nutrition Paper, No. 52, Add. 11, 2003.
169. Evaluation of certain veterinary drug residues in food (Sixty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 925, 2004.
170. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/16, 2004.
171. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 53, 2005.
172. Compendium of food additive specifications: addendum 12. FAO Food and Nutrition Paper, No. 52, Add. 12, 2004.
173. Evaluation of certain food additives (Sixty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 928, 2005.
174. Safety evaluation of certain food additives. WHO Food Additives Series, No. 54, 2005.
175. Compendium of food additive specifications: addendum 13. FAO Food and Nutrition Paper, No. 52, Add. 13 (with Errata), 2005.
176. Evaluation of certain food contaminants (Sixty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 930, 2005.
177. Safety evaluation of certain contaminants in food. WHO Food Additives Series, No. 55/FAO Food and Nutrition Paper, No. 82, 2006.
178. Evaluation of certain food additives (Sixty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 934, 2006.
179. Safety evaluation of certain food additives. WHO Food Additives Series, No. 56, 2006.
180. Combined compendium of food additive specifications. FAO JECFA Monographs 1, Volumes 1–4, 2005, 2006.
181. Evaluation of certain veterinary drug residues in food (Sixty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 939, 2006.
182. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs 2, 2006.
183. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 57, 2006.
184. Evaluation of certain food additives and contaminants (Sixty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 940, 2007.
185. Compendium of food additive specifications. FAO JECFA Monographs 3, 2006.
186. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 58, 2007.
187. Evaluation of certain food additives and contaminants (Sixty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 947, 2007.

188. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 59, 2008.
189. Compendium of food additive specifications. FAO JECFA Monographs 4, 2007.
190. Evaluation of certain food additives (Sixty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 952, 2009.
191. Safety evaluation of certain food additives. WHO Food Additives Series, No. 60, 2009.
192. Compendium of food additive specifications. FAO JECFA Monographs 5, 2009.
193. Evaluation of certain veterinary drug residues in food (Seventieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 954, 2009.
194. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 61, 2009.
195. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs 6, 2009.
196. Evaluation of certain food additives (Seventy-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 956, 2010.
197. Safety evaluation of certain food additives. WHO Food Additives Series, No. 62, 2010.
198. Compendium of food additive specifications. FAO JECFA Monographs 7, 2009.
199. Evaluation of certain contaminants in food (Seventy-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 959, 2011.
200. Safety evaluation of certain contaminants in food. WHO Food Additives Series, No. 63/FAO JECFA Monographs 8, 2011.
201. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs 9, 2010.
202. Evaluation of certain food additives and contaminants (Seventy-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 960, 2011.
203. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 64, 2011.
204. Compendium of food additive specifications. FAO JECFA Monographs 10, 2010.
205. Evaluation of certain food additives and contaminants (Seventy-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 966, 2011.
206. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 65, 2011.
207. Compendium of food additive specifications. FAO JECFA Monographs 11, 2011.
208. Evaluation of certain veterinary drug residues in food (Seventy-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 969, 2012.
209. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 66, 2012.
210. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs 12, 2012.
211. Evaluation of certain food additives (Seventy-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 974, 2012.
212. Safety evaluation of certain food additives. WHO Food Additives Series, No. 67, 2012.

213. Compendium of food additive specifications. FAO JECFA Monographs 13, 2012.
214. Evaluation of certain food additives and contaminants (Seventy-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 983, 2013.
215. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 68, 2013.
216. Compendium of food additive specifications. FAO JECFA Monographs 14, 2013.
217. Evaluation of certain veterinary drug residues in food (Seventy-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 988, 2014.
218. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 69, 2014.
219. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs 15, 2014.
220. Evaluation of certain food additives (Seventy-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 990, 2015.
221. Safety evaluation of certain food additives. WHO Food Additives Series, No. 70, 2015.
222. Compendium of food additive specifications. FAO JECFA Monographs 16, 2014.
223. Evaluation of certain food additives and contaminants (Eightieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 995, 2016.
224. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 71, 2015.
225. Compendium of food additive specifications. FAO JECFA Monographs 17, 2015.
226. Evaluation of certain veterinary drug residues in food (Eighty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 997, 2016.
227. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 72, 2016.
228. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs 18, 2016.
229. Safety evaluation of certain food additives and contaminants. Supplement 1: Non-dioxin-like polychlorinated biphenyls. WHO Food Additives Series, No. 71-1, 2016.
230. Evaluation of certain food additives (Eighty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 1000, 2016.
231. Compendium of food additive specifications. FAO JECFA Monographs 19, 2016.
232. Safety evaluation of certain food additives. WHO Food Additives Series, No. 73, 2017.
233. Evaluation of certain contaminants in food (Eighty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 1002, 2017.
234. Evaluation of certain food additives (Eighty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 1007, 2017.
235. Safety evaluation of certain contaminants in food. WHO Food Additives Series, No. 74, 2018 (in press).
236. Compendium of food additive specifications. FAO JECFA Monographs 20, 2017.
237. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 75, 2018 (in press).

238. Evaluation of certain veterinary drug residues in food (Eighty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 1008, 2018.
239. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs 21, 2018.
240. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 76, 2019 (in press).



ANNEX 2

Toxicological information and information on specifications

Food additives evaluated toxicologically and assessed for dietary exposure

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions
Anionic methacrylate copolymer (AMC)	N, T ^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-Pentanylfuran	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-(2 <i>H</i>)-fur-4-yl butyrate	93%	4-Hydroxy-2,5-dimethyl-3(2 <i>H</i>)-furanone (1–3%) Butyric acid (No. 87) (1–3%)	The SPET value for No. 1519 is 1 200 µg/day, and 3% of this value is 36 µg/day, which is below the class III threshold of toxicological concern. Butyric acid (No. 87) has previously been evaluated by the Committee to be of no safety concern at estimated dietary exposures when used as a flavouring agent.
1524	Furfuryl 2-methyl-3-furyl disulfide	90%	Di-(2-methyl-3-furyl) disulfide (6–7%)	The SPET value for No. 1524 is 10 µg/day, and 7% of this value is 0.7 µg/day, which is below the class III threshold of toxicological concern. The major secondary component of No. 1524 is therefore not considered to present a safety concern at estimated dietary exposures from the use of No. 1524 as a flavouring agent.
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
WHO Press, World Health Organization ■ 1211 Geneva 27, Switzerland ■ www.who.int/bookorders
tel.: +41 22 791 3264; **fax:** +41 22 791 4857; **email:** bookorders@who.int

Evaluation of certain food additives

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



9 789241 210232

SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006

Version 6.3

Revision Date 14.04.2022

Print Date 20.11.2023

GENERIC EU MSDS - NO COUNTRY SPECIFIC DATA - NO OEL DATA

SECTION 1: Identification of the substance/mixture and of the company/undertaking

1.1 Product identifiers

Product name : Dextrin, from corn
Dextrin from corn

Product Number : D2006

Brand : Sigma

REACH No. : A registration number is not available for this substance as the substance or its uses are exempted from registration, the annual tonnage does not require a registration or the registration is envisaged for a later registration deadline.

CAS-No. : 9004-53-9

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company : Merck Life Science Sp.z.o.o.
Szelągowska 30
PL-61-626 POZNAN

Telephone : +48 61 8290-100

Fax : +48 61 8290-120

E-mail address : TechnicalService@merckgroup.com

1.4 Emergency telephone

Emergency Phone # : +(48)-223988029 (CHEMTREC) 112
(numer alarmowy)

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

Not a hazardous substance or mixture according to Regulation (EC) No 1272/2008.

2.2 Label elements

Not a hazardous substance or mixture according to Regulation (EC) No 1272/2008.

2.3 Other hazards

This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.



SECTION 3: Composition/information on ingredients

3.1 Substances

Formula : C₆H₁₂O₆
CAS-No. : 9004-53-9
EC-No. : 232-675-4

No components need to be disclosed according to the applicable regulations.

SECTION 4: First aid measures

4.1 Description of first-aid measures

If inhaled

If breathed in, move person into fresh air. If not breathing, give artificial respiration.

In case of skin contact

Wash off with soap and plenty of water.

In case of eye contact

Flush eyes with water as a precaution.

If swallowed

Never give anything by mouth to an unconscious person. Rinse mouth with water.

4.2 Most important symptoms and effects, both acute and delayed

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed

No data available

SECTION 5: Firefighting measures

5.1 Extinguishing media

Suitable extinguishing media

Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

5.2 Special hazards arising from the substance or mixture

Carbon oxides

5.3 Advice for firefighters

Wear self-contained breathing apparatus for firefighting if necessary.

5.4 Further information

No data available

SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures

Avoid dust formation. Avoid breathing vapors, mist or gas.

For personal protection see section 8.



6.2 Environmental precautions

No special environmental precautions required.

6.3 Methods and materials for containment and cleaning up

Sweep up and shovel. Keep in suitable, closed containers for disposal.

6.4 Reference to other sections

For disposal see section 13.

SECTION 7: Handling and storage

7.1 Precautions for safe handling

Advice on protection against fire and explosion

Provide appropriate exhaust ventilation at places where dust is formed.

Hygiene measures

General industrial hygiene practice.

For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities

Storage conditions

Keep container tightly closed in a dry and well-ventilated place. Store in cool place.

Hygroscopic.

Storage class

Storage class (TRGS 510): 13: Non Combustible Solids

7.3 Specific end use(s)

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

SECTION 8: Exposure controls/personal protection

8.1 Control parameters

Ingredients with workplace control parameters

8.2 Exposure controls

Personal protective equipment

Eye/face protection

Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin protection

Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

The selected protective gloves have to satisfy the specifications of Regulation (EU) 2016/425 and the standard EN 374 derived from it.

Body Protection

Choose body protection in relation to its type, to the concentration and amount of dangerous substances, and to the specific work-place., The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.



Respiratory protection

Respiratory protection is not required. Where protection from nuisance levels of dusts are desired, use type N95 (US) or type P1 (EN 143) dust masks. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Control of environmental exposure

No special environmental precautions required.

SECTION 9: Physical and chemical properties

9.1 Information on basic physical and chemical properties

a) Physical state	powder
b) Color	light yellow
c) Odor	No data available
d) Melting point/freezing point	No data available
e) Initial boiling point and boiling range	No data available
f) Flammability (solid, gas)	May form combustible dust concentrations in air.
g) Upper/lower flammability or explosive limits	No data available
h) Flash point	No data available
i) Autoignition temperature	No data available
j) Decomposition temperature	No data available
k) pH	No data available
l) Viscosity	Viscosity, kinematic: No data available Viscosity, dynamic: No data available
m) Water solubility	No data available
n) Partition coefficient: n-octanol/water	No data available
o) Vapor pressure	No data available
p) Density	No data available
Relative density	No data available
q) Relative vapor density	No data available
r) Particle characteristics	No data available
s) Explosive properties	No data available



t) Oxidizing properties No data available

9.2 Other safety information

No data available

SECTION 10: Stability and reactivity

10.1 Reactivity

No data available

10.2 Chemical stability

Stable under recommended storage conditions.

10.3 Possibility of hazardous reactions

No data available

10.4 Conditions to avoid

Exposure to moisture may affect product quality.

10.5 Incompatible materials

Strong oxidizing agents

10.6 Hazardous decomposition products

In the event of fire: see section 5

SECTION 11: Toxicological information

11.1 Information on toxicological effects

Acute toxicity

Oral: No data available

Inhalation: No data available

Dermal: No data available

Skin corrosion/irritation

No data available

Serious eye damage/eye irritation

No data available

Respiratory or skin sensitization

No data available

Germ cell mutagenicity

No data available

Carcinogenicity

No data available

Reproductive toxicity

No data available

Specific target organ toxicity - single exposure

No data available

Specific target organ toxicity - repeated exposure

No data available

Aspiration hazard

No data available



11.2 Additional Information

RTECS: HH9450000

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

SECTION 12: Ecological information

12.1 Toxicity

No data available

12.2 Persistence and degradability

No data available

12.3 Bioaccumulative potential

No data available

12.4 Mobility in soil

No data available

12.5 Results of PBT and vPvB assessment

This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

12.6 Endocrine disrupting properties

No data available

12.7 Other adverse effects

No data available

SECTION 13: Disposal considerations

13.1 Waste treatment methods

Product

Offer surplus and non-recyclable solutions to a licensed disposal company.

Contaminated packaging

Dispose of as unused product.

SECTION 14: Transport information

14.1 UN number

ADR/RID: -

IMDG: -

IATA: -

14.2 UN proper shipping name

ADR/RID: Not dangerous goods

IMDG: Not dangerous goods

IATA: Not dangerous goods

14.3 Transport hazard class(es)

ADR/RID: -

IMDG: -

IATA: -

14.4 Packaging group

ADR/RID: -

IMDG: -

IATA: -



14.5 Environmental hazards

ADR/RID: no

IMDG Marine pollutant: no

IATA: no

14.6 Special precautions for user

Further information

Not classified as dangerous in the meaning of transport regulations.

SECTION 15: Regulatory information

15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture

This material safety data sheet complies with the requirements of Regulation (EC) No. 1907/2006.

15.2 Chemical Safety Assessment

For this product a chemical safety assessment was not carried out

SECTION 16: Other information

Further information

Copyright 2020 Sigma-Aldrich Co. LLC. License granted to make unlimited paper copies for internal use only.

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Corporation and its Affiliates shall not be held liable for any damage resulting from handling or from contact with the above product. See www.sigma-aldrich.com and/or the reverse side of invoice or packing slip for additional terms and conditions of sale.

The branding on the header and/or footer of this document may temporarily not visually match the product purchased as we transition our branding. However, all of the information in the document regarding the product remains unchanged and matches the product ordered. For further information please contact mlsbranding@sial.com.

