Toxicological profile for

Gum arabic

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

Not applicable.

1.2. Synonyms

Acacia Gum; Arabic, Gum; Gum Acacia; Gum Arabic; Gum, Acacia (PubChem)

1.3. Molecular formula

C12H36 (PubChem)

1.4. Structural Formula

No data available to us at this time.

1.5. Molecular weight (g/mol)

240,000 (HSDB, 2002); 240,000-580,000 (Merck, 2013); approximately 350,000 (EFSA, 2019); 180.41(PubChem)

1.6. CAS registration number

9000-01-5

1.7. Properties

1.7.1. Melting point

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

1.7.2. Boiling point

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

1.7.3. Solubility

Completely soluble in hot and cold water; yielding a viscous solution of mucilage; insoluble in alcohol (HSDB, 2002); Almost completely soluble in twice its weight of water, saturated solutions 37g/100 ml water at 25°C (Merck, 2013), 50 to 100 mg/mL at 64°, 1 g dissolves in 2 ml of cold water forming a solution which flows readily and is acid to litmus (PubChem, 2023)

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): 90C (GESTIS)

1.7.9. Stability

“Stable in acid conditions and also has excellent heat stability” (EFSA, 2019)

1.7.10. Vapor pressure

No data available to us at this time.

1.7.11. log Kow

No data available to us at this time.

2. General information

2.1. Exposure

“USE: As mucilage, excipient for tablets, size, emulsifier, thickener, also in candy, other foods; as colloidal stabilizer. In the manufacture of spray-dried “fixed” flavors – stable, powdered flavors used in packaged dry-mix products (puddings, desserts, cake mixes) where flavor stability and long shelf life are important”

As taken from Merck, 2013.

“Acacia is used in pharmaceutical manufacturing as a suspending and emulsifying agent, as a tablet binder, and in pastilles. It is used as an emulsifier and stabiliser in the food industry”

As taken from Martindale, 1999.

Gum arabic (Acacia) is listed as an ingredient in personal care products (at 0.1-1% where specified) by the CPID.

Acacia Senegal gum (CAS RN 9000-01-5) is used as a film forming and fragrance ingredient and in cosmetics in the EU. As taken from CosIng.

Acacia gum is listed as a fragrance ingredient by International Fragrance List (IFRA).

“Acacia gum (E414) (EFSA-Q-2011-00513): application of the decision tree on the risk assessment of food additives with “no numerical ADI and generally authorized at QS use”:

….Because the gum is authorized at QS use, in absence of use data no exposure evaluation was available. However a specific call for data is being launched (batch 3) with the aim of collecting uses and use levels data.”

As taken from EFSA, 2014.

Gum arabic (CAS RN 9000-01-5) is listed as an approved ingredient in food and non-food use pesticide products in the US EPA InertFinder Database.

Acacia (no CAS RN listed) is used as a binder, delivery system, emulsifying agent, gelling agent, polishing agent, stabilizing agent and thickening agent, and acacia Senegal gum (no CAS RN listed) is used as a as an adhesive, controlled release vehicle, delivery system, emulsifying agent, flavour enhancer, fragrance ingredient, stabilizing agent and thickening agent in non-medicinal natural health products. The dried gum and gummy exudate of acacia arabica (no CAS RN) are also listed as homeopathic substances on Health Canada's Natural Health Products Database (Health Canada, 2021).

Gum arabic (Acacia gum) is a food additive that is included in "Codex General Standard for Food Additives" (GSFA) of Codex Alimentarius Functional classes for gum arabic are Bulking agent, Carrier, Emulsifier, Glazing agent, Stabilizer, Thickener. (Codex Alimentarius, FAO, 2019)

2.2. Combustion products

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

Ingredient CAS number	Max. cig. appln. level (ppm)	Composition of pyrolysate (Compound, %)	Max. smoke (µg)
Acacia gum CAS 9000-01-5	15000	Acetol 20.7 Acetic acid 7.5 Xanthosine 5.0 Benzenediol 4.7 Furfural 3.2 2-Butanone 0.5	1550 560 380 380 240 38

2.3. Ingredient(s) from which it originates

Acacia-derived cosmetic ingredient terminology, description, and function (Continued)

1995-1997 Terminology (Wenninger and McEwen 1995, 1997)				2004 Terminology (Gottschalck and McEwen 2004)		
Name	Description	Cosmetics function		Name	Description	Cosmetics function
Acacia Senegal	Plant material derived from the dried, gummy exudate of the acacia, Acacia senegal	Not reported		Acacia Senegal Gum	Plant material derived from the dried, gummy exudate of the acacia, Acacia senegal	Not reported
Acacia Senegal Extract	Extract of the flowers and stems of the acacia, Acacia senegal	Biological additive		Acacia Senegal Extract	Extract of the flowers and stems of the acacia, Acacia senegal	Not reported
Acacia Senegal Gum Extract	Extract of the gum of the acacia, Acacia senegal	Biological additive		Acacia Senegal Gum Extract	Extract of the gum of the acacia, Acacia senegal	Not reported

As taken from CIR, 2005.

Gum Arabic is obtained from trees of the genus acacia... Gum arabic is the result of an infection, either bacterial or fungoid. It is exuded only by unhealthy trees; heat, poor nutrition, and drought stimulate its production. ...infection takes place through wounds in the tree... [Furia, T.E. (Ed.). CRC Handbook of Food Additives 2nd ed. Cleveland: The Chemical Rubber Co., 1972. p. 312] **PEER REVIEWED**

...USP acacia is dried gummy exudation from stems and branches of Acacia senegal (l) Willd, Leguminosae, or other African species of Acacia. According to CL Mantell, the Water-Sol Gums (NY, 1947), Kordofan gum... from Acacia verek...from Kordofan province (Sudan) is considered best commercial variety. [Budavari, S. (Ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Whitehouse Station, NJ: Merck and Co., Inc., 1996. p. 3] **PEER REVIEWED**

As taken from HSDB, 2002.

“Powdered exudate from various Acacia species, especially A. senegal (Leguminosae).”

As taken from PubChem.

3. Status in legislation and other official guidance

FIFRA Requirements:

Residues of gum arabic are exempted from the requirement of a tolerance when used as a surfactant, suspending agent and dispersing agent in accordance with good agricultural practices as inert (or occasionally active) ingredients in pesticide formulations applied to growing crops or to raw agricultural commodities after harvest. [40 CFR 180.1001(c) (7/1/99)] **PEER REVIEWED**

FDA Requirements:

Substance added directly to human food affirmed as generally recognized as safe (GRAS). [21 CFR 184.1330 (4/1/99)] **PEER REVIEWED**

Gum arabic used as a stabilizer in animal drugs, feeds, and related products is generally recognized as safe when used in accordance with good manufacturing or feeding practice. [21 CFR 582.7330 (4/1/99)] **PEER REVIEWED**

Manufacturers, packers, and distributors of drug and drug products for human use are responsible for complying with the labeling, certification, and usage requirements as prescribed by the Federal Food, Drug, and Cosmetic Act, as amended (secs 201-902, 52 Stat. 1040 et seq., as amended; 21 U.S.C. 321-392). [21 CFR 200-299, 300-499, 820, and 860 (4/1/99)] **PEER REVIEWED**

As taken from HSDB, 2002.

Based on the lack of adverse effects in the available toxicity studies, JECFA in 1982 and in 1990 allocated an ADI ‘not specified’ to gum acacia. The Committee stressed that the evaluation covered only gum acacia from Acacia senegal and closely related species. In 1998 (51st session), the specification was changed to cover also gum acacia from Acacia seyal (JECFA, 1982a; 1990; 1998).

As taken from EFSA, 2010a

The EFSA Panel concluded that there is no need for a numerical ADI for acacia gum (E 414) (a conclusion that is not applicable for infants under the age of 12 weeks).

As taken from EFSA, 2017

“Concentration of use data from industry (CTFA 2000a) shows the highest concentration of Acacia Senegal Gum (9%) in shampoos. Acacia Senegal Gum Extract was reported at a concentration of 0.001% in bath soaps and detergents. For many uses of these ingredients, information regarding

use concentration for specific product categories is provided, but the number of such products is not known, but they must be assumed to be in use."

As taken from CIR, 2005.

Acacia, gum (Acacia senegal (L.) Willd.) is included on the FDA's list of Substances Added to Food (formerly EAFUS) as an emulsifier or emulsifier salt, flavor enhancer, formulation aid, processing aid, propellant, solvent or vehicle, stabilizer or thickener, surface-active agent and texturizer and is covered under the following Code of Federal Regulations, Title 21:

FDA PART 169 -- Food Dressings and Flavorings
Subpart B--Requirements for Specific Standardized Food Dressings and Flavorings
Sec.169.179 Vanilla powder

FDA PART 172 -- Food Additives Permitted For Direct Addition To Food For Human Consumption
Subpart H--Other Specific Usage Additives
Sec. 172.780 Acacia (Gum Arabic)

FDA PART 184 -- Direct Food Substances Affirmed As Generally Recognized As Safe
Subpart B--Listing of Specific Substances Affirmed as GRAS
Sec. 184.1330 Acacia (Gum Arabic)

As taken from FDA, 2024a

Gum Arabic is not registered under REACH (ECHA).

Gum Arabic is not classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2023).

"Acacia gum (E414) (EFSA-Q-2011-00513): application of the decision tree on the risk assessment of food additives with "no numerical ADI and generally authorized at QS use":

….Acacia gum was shown to be not genotoxic and neither carcinogenic potential nor reproductive and developmental toxic effects have been observed. Further to sub-chronic studies in rodents a NOAEL of 5000 mg/kg bw/day has been identified and used as a point of departure (POD). Applying the appropriate UF an ADI of 25 mg/kg bw/day could be derived. However in case of food additives characterized by "low intrinsic toxicity" and are authorized at QS, the decision to allocate an ADI should be further discussed."

As taken from EFSA, 2014.

Gum arabic (CAS RN 9000-01-5) is approved for food and non-food use pesticide products (InertFinder).

Gum arabic is listed in the US EPA Toxic Substances Control Act (TSCA) inventory and also in the US EPA 2024 CDR and 2024 CDR Full Exempt (Chemical Data Reporting) lists.

US EPA Substance Registry Services (SRS)

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

GUM ARABIC

Synonyms:	ACACIA GUM, ACACIA SENEGAL, ACACIA SEYAL, ARABIC GUM
CAS number:	9000-01-5

INS:	414
Functional Class:	Food Additives o EMULSIFIER o STABILIZER THICKENER

Evaluations

Evaluation year:	1989
ADI:	NOT SPECIFIED
Meeting:	49
Specs Code:	R (1997)
Report:	TRS 789-JECFA 35/24
Tox Monograph:	FAS 26-JECFA 35/77
Specification:	COMPENDIUM ADDENDUM 6/FNP 52 Add.6/69 (1998). R; FAO JECFA Monographs 1 vol.2/145
Previous Years:	1997, COMPENDIUM ADDENDUM 5/FNP 52 Add.5/53. R 1995, COMPENDIUM ADDENDUM 3/FNP 52 Add.3/83. R 1989, FNP 49-JECFA 35/23; COMPENDIUM/735. R 1984, FNP 34-JECFA 29/93. R 1982, TRS 683-JECFA 26/28, FNP 25-JECFA 26/93, FAS 17-JECFA 26/50. ADI NOT SPECIFIED.

As taken from JECFA, 2010

Gum Arabic (acacia gum, E414) is authorised for use as a food additive in the EU under legislation (EU) nos 1129/2011 and as a component of Group I, Additives, under 1129/2011, 438/2013, 2015/647, 2015/1832 and 2018/1497 (European Commission).

Acacia gum (Acacia senegal (l.) Willd.) has been given GRAS (generally recognized as safe) status by FEMA (FEMA no. 2001) (Hall and Oser, 1965).

Acacia syrup and acacia are included on the US FDA's list of inactive ingredients for approved drug products. They are permitted for use as ingredients in various products, at the following maximum potencies per unit dose and maximum daily exposures:

Inactive Ingredient	Route	Dosage Form	CAS Number	UNII	Maximum Potency per unit dose	Maximum Daily Exposure (MDE)
ACACIA	BUCCAL	GUM, CHEWING	9000015	5C5403N26O		280mg
ACACIA	BUCCAL	TABLET	9000015	5C5403N26O	9.1mg	
ACACIA	INTRAMUSCULAR	SUSPENSION	9000015	5C5403N26O	1mg	
ACACIA	ORAL	CAPSULE	9000015	5C5403N26O		64mg

ACACIA	ORAL	CAPSULE, EXTENDED RELEASE	9000015	5C5403N26O		51mg
ACACIA	ORAL	LOZENGE	9000015	5C5403N26O		108mg
ACACIA	ORAL	POWDER	9000015	5C5403N26O	800mg	
ACACIA	ORAL	POWDER, FOR SOLUTION	9000015	5C5403N26O	NA	
ACACIA	ORAL	POWDER, FOR SUSPENSION	9000015	5C5403N26O		7386mg
ACACIA	ORAL	SUSPENSION	9000015	5C5403N26O		2mg
ACACIA	ORAL	SYRUP	9000015	5C5403N26O	NA	
ACACIA	ORAL	TABLET	9000015	5C5403N26O	70mg	
ACACIA	ORAL	TABLET, CHEWABLE	9000015	5C5403N26O		121mg
ACACIA	ORAL	TABLET, COATED	9000015	5C5403N26O	156mg	
ACACIA	ORAL	TABLET, DELAYED RELEASE	9000015	5C5403N26O	30mg	
ACACIA	ORAL	TABLET, EXTENDED RELEASE	9000015	5C5403N26O	34.4mg	
ACACIA	ORAL	TABLET, FILM COATED	9000015	5C5403N26O	0.14mg	
ACACIA	ORAL	TABLET, ORALLY DISINTEGRATING	9000015	5C5403N26O		7mg
ACACIA	SUBLINGUAL	TABLET	9000015	5C5403N26O	9.1mg	

As taken from FDA, 2024b

Gum arabic (CAS RN 9000-01-5) is included on the US EPA's Safer Chemical Ingredients List and it's marked on green circle. (US EPA, 2024).

Gum arabic (CAS RN 9000-01-5) "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, 2017).

"As follow-up to the assessment [see EFSA, 2017], the Panel on Food Additives and Flavourings (FAF) was requested to assess the safety of acacia gum (E 414) as carry-over in food for infants below 16 weeks of age belonging to food categories 13.1.1 (Infant formulae) and 13.1.5.1 (Dietary foods for infants for special medical purposes and special formulae for infants) and to address the issues already identified during the re-evaluation of the food additive when used in food for the general population."

"Taking the highest doses tested without adverse effects in subchronic studies of 5,000 mg acacia gum/kg bw per day in rat and 20,000 mg acacia gum/kg bw per day in mice from the former EFSA evaluation in 2017 and comparing them with the exposure in infants of 2.6 mg/kg bw per day (high

level estimate), MOS are roughly 2,000 and 8,000. These large MOS indicate that there is no reason for health concern."

"Based on the analytical data submitted in response to this call, the Panel recommended to lower the limits in the specifications for toxic elements and identified the need for further specifications for aluminium, microbiological criteria and protein residues. The Panel noted that information was not provided for oxidising enzymes and recommended that oxidases and peroxidases should be inactivated during the manufacturing process."

"According to Regulation (EC) No 1333/2008 (Annex III, part 5, section B), acacia gum (E 414) is authorised for use as a food additive in nutrient preparations intended to be used in foodstuffs for infants and young children, including food for infants below 16 weeks of age."

E number	Name of the food additive	Maximum permitted level	Nutrient to which the food additive may be added	Food category
E 414	Acacia gum	150,000 mg/kg in the nutrient preparation and 10 mg/kg carryover in final products	All nutrients	Foods for infants and young children

"Acacia gum has also been reviewed by the Nordic Council of Ministers (TemaNord, 2002), who concluded that even though the existing data do not raise any toxicological concern, allergy/intolerance and the problem of marketing gums originating from acacia species not included in their evaluation should be considered in future evaluations."

As taken from EFSA, 2019.

Acacia (no CAS RN listed) is included on Health Canada's Natural Health Products Ingredients Database and is classified as an NHP for medicinal use under Schedule 1, item 2 (extract) of the Natural Health Products Regulations (Health Canada, 2021).

4. Metabolism/Pharmacokinetics

4.1. Metabolism/metabolites

Metabolic studies are limited, but it has been demonstrated in the rat that this gum is completely metabolized when it comprises less than 10% of the diet.

As taken from JECFA, 1982

"The in vitro degradation and the in vivo digestibility of acacia gum have been investigated in animals and humans models and in a human study. The Panel considered that these data indicated that acacia gum would be not absorbed intact but fermented by enteric bacteria in humans. The rate of hydrolysis in the gastrointestinal tract in humans is unknown; however, the Panel considered that acacia gum is unlikely to be absorbed intact, and that the limited extent of its fermentation would lead to products such as short-chain fatty acids (SCFA)."

As taken from EFSA, 2017

"Gum arabic has been found to be fermented mainly to acetate, propionate and butyrate, which can be absorbed and metabolized by normal metabolic pathways. Short chain fatty acids have been found to significantly affect intestinal and liver metabolism as sources of energy or metabolic effectors (Ali, Ziada & Blunden, 2009)."

"Ross et al. (1983) [probably involving daily ingestion by adults of perhaps up to 30 g over around 20 days] found that gum arabic could not be detected in the stool, indicating complete fermentation in the colon."

As taken from JECFA, 2019.

4.2. Absorption, distribution and excretion

Recent Studies Demonstrated That Acacia Is Stored In Vacuoles For Long Periods By The Liver.[Thienes, C., and T.J. Haley Clinical Toxicology 5th ed. Philadelphia: Lea and Febiger, 1972. p. 146] **PEER REVIEWED**

As taken from HSDB, 2002.

“At dietary levels of less than 10%, arabic gum is fully absorbed with a caloric equivalent of 4 calories per gram (Shue et al., 1962).”

As taken from JECFA, 1982.

A study of the effects of dietary gum arabic in the rat (Abstract)

Gum arabic (GA) is a water-soluble polysaccharide (molecular weight approximately 850 000) containing rhamnose, arabinose, glucuronic acid and galactose. The metabolism of GA has been studied in the rat. Adult male Wistar rats were given GA incorporated into either an Oxoid breeders (OB) diet or an elemental (Elem) diet. Intestinal contents were examined for precipitable GA using acidified ethanol. GA was found from stomach to small intestine but not in the caecum, colon or rectum. Caecal excision and restoration of intestinal continuity resulted in GA recovery from stomach to rectum. Excreted methane, hydrogen and volatile fatty acids (VFA) were measured as indicators of bacterial activity in the caecum and colon. Methane excretion increased on the OB + GA diet and H2 concentrations remained unaltered. The Elem diet abolished gas production. When the animals were given the Elem + GA diet, H2 and methane were only produced after 28 d. Faecal VFA increased with increasing GA intake, acetate concentration increased and butyrate concentration decreased with increasing GA dosage. Significant decreases in concentrations of VFA were found from caecum to left colon and from left colon to faeces. It can be concluded that GA degradation occurs in the caecum and is associated with increased methane excretion, increased VFA concentrations and changes in the proportions of various VFA in the faeces. As taken from McLean Ross AH et al. (1984)

EFSA documented stated ‘literature show that arabic gum (i.e. gum acacia) is almost completely digested by guinea-pigs (O'Dell et al., 1957). Studies in the rat show that gum Arabic degradation occurs in the caecum and that it is associated with increased methane excretion, increased volatile fatty acids (VFA) concentration and changes in the proportions of various VFAs in the feces (Ross et al, 1981). In a study in humans, gum arabic administered to men for 21 days at a dose of about 350 mg/kg bw/day, had little effect on glucose tolerance and stool weight, but decreased the serum cholesterol. There was no significant increase in fecal bile acids and neutral sterols. Gum Arabic could not be recovered from the stool, which according to the authors suggests that gum arabic is metabolized in the colon (Ross et al., 1983’), (EFSA, 2010a).

“The in vitro degradation and the in vivo digestibility of acacia gum have been investigated in animals and humans models and in a human study. The Panel considered that these data indicated that acacia gum would be not absorbed intact but fermented by enteric bacteria in humans. The rate of hydrolysis in the gastrointestinal tract in humans is unknown; however, the Panel considered that acacia gum is unlikely to be absorbed intact, and that the limited extent of its fermentation would lead to products such as short-chain fatty acids (SCFA).”

As taken from EFSA, 2017

4.3. Interactions

“Non-covalent interaction of alcohol dehydrogenase with polysaccharides was studied using three neutral and three anionic polysaccharides. The process of interaction of alcohol dehydrogenase with gum Arabic was optimized with respect to the ratio of enzyme to gum Arabic, pH, and molarity

of buffer. Alcohol dehydrogenase-gum Arabiccomplex formed under optimized conditions showed 93% retention of original activity with enhanced thermal and pH stability. Lower inactivation rate constant of alcohol dehydrogenase-gum Arabic complex within the temperature range of 45 to 60 °C implied its better stability. Half-life of alcohol dehydrogenase-gum Arabic complex was higher than that of free alcohol dehydrogenase. A slight increment was observed in kinetic constants (K(m) and V(max)) of gum Arabic-complexed alcohol dehydrogenase which may be due to interference by gum Arabic for the binding of substrate to the enzyme. Helix to turn conversion was observed in complexed alcohol dehydrogenase as compared to free alcohol dehydrogenase which may be responsible for observed stability enhancement." As taken from Jadhav SB et al. 2014. Appl. Microbiol. Biotechnol. 98(14), 6307-16. PubMed, 2015 available at http://www.ncbi.nlm.nih.gov/pubmed/24658590

"Pathogenesis of adenine-induced chronic renal failure may involve inflammatory, immunological and/or oxidant mechanisms. Gum arabic (GA) is a complex polysaccharide that acts as an anti-oxidant which can modulate inflammatory and/or immunological processes. Therefore, we tested here the effect of GA treatment (15 % in the drinking water for 4 weeks) in plasma and urine of rats, on a novel cytokine that has been shown to be pro-inflammatory, viz, DNA-binding high-mobility group box-1 protein (HMGB1). Adenine (0.75 % in the feed, 4 weeks) significantly increased indoxyl sulphate, urea and creatinine concentrations in plasma, and significantly decreased the creatinine clearance. GA significantly abated these effects. The concentrations of HMGB1 in urine before the start of the experiment were similar in all four groups. However, 24 h after the last treatment, adenine treatment increased significantly the concentration of HMGB1 when compared with the control. GA treatment did not affect the HMGB1 concentration in urine. Moreover, the concentration of HMGB1 in plasma obtained 24 h after the last treatment in rats treated with adenine was drastically reduced compared with the control group. This may explain its significant rise in urine. In conclusion, HMGB1 can be considered a potentially useful biomarker in adenine induced CRF and its treatment." As taken from Ali BH et al. 2015. Physiol. Res. 64(1), 147-51. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/25194125

"Thaumatin is a sweetener and flavor modifier commonly used in the food industry. Likewise, gum arabic is widely used as a food stabilizer and thickening agent. We report here that a powder mixture composed of 10% thaumatin and 90% gum arabic led to allergic symptoms in the upper airways in occupationally exposed individuals: four of eight workers of a chewing gum factory exposed to this powder mixture had pronounced rhinitis. A positive skin prick test result for pure thaumatin was obtained in all four individuals with rhinitis of whom two also had a positive skin prick test result for pure gum arabic and gum arabic-specific IgE. Subsitution of a powdered thaumatin with a liquid form reduced symptoms among the rhinitic workers. Although gum arabic is a well-known potential allergen, we were unable to find prior documentation of allergic symptoms to thaumatin when it is used in the food industry." As taken from Tschannen MP et al. 2017. Am. J. Ind. Med. 60(7), 664-669. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28543634

"This study assessed the potential adverse health effects of long-term low-dose exposure to chemical mixtures simulating complex real-life human exposures. Four groups of Sprague Dawley rats were administered mixtures containing carbaryl, dimethoate, glyphosate, methomyl, methyl parathion, triadimefon, aspartame, sodium benzoate, calcium disodium ethylene diamine tetra-acetate, ethylparaben, butylparaben, bisphenol A, and acacia gum at doses of 0, 0.25, 1 or 5 times the respective Toxicological Reference Values (TRV): acceptable daily intake (ADI) or tolerable daily intake (TDI) in a 24 weeks toxicity study. Body weight gain, feed and water consumption were evaluated weekly. At 24 weeks blood was collected and biochemistry parameters and redox status markers were assessed. Adverse effects were observed on body weight gain and in hepatotoxic parameters such as the total bilirubin, alanine aminotransferase (ALT) and alkaline phosphatase (ALP), especially in low dose and affecting mainly male rats. The low dose group showed increased catalase activity both in females and males, whereas the high dose group exhibited decreased protein carbonyl and total antioxidant capacity (TAC) levels in both sex groups. Non-monotonic

effects and adaptive responses on liver function tests and redox status, leading to non-linear dose-responses curves, are probably produced by modulation of different mechanisms." As taken from Docea AO et al. 2018. Food Chem. Toxicol. 115, 470-481. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29621577

"Water-pipe smoking (WPS) is prevalent in the East and elsewhere. WPS exposure is known to induce thrombosis and cardiovascular toxicity involving inflammation and oxidative stress. Here, we have investigated the effect of Gum Arabic (GA), a prebiotic with anti-oxidant, anti-inflammatory and cytoprotective properties, on WPS exposure (30 min/day for 1 month) on coagulation and cardiac homeostasis, and their possible underlying mechanisms in mice. Animals received either GA in drinking water (15%, w/v) or water only for the entire duration of study. GA significantly mitigated thrombosis in pial microvessels in vivo, platelet aggregation in vitro, and the shortening of prothrombin time induced by WPS exposure. The increase in plasma concentrations of fibrinogen, plasminogen activator inhibitor-1 and markers of lipid peroxidation, 8-isoprostane and malondialdehyde, induced by WPS were significantly reduced by GA administration. Moreover, WPS exposure induced a significant increase in systolic blood pressure and the concentrations of the pro-inflammatory cytokines tumor necrosis factor-α and interleukin 1β in heart homogenates. GA significantly alleviated these effects, and prevented the decrease of reduced glutathione, catalase and total nitric oxide levels in heart homogenates. Immunohistochemical analysis of the hearts showed that WPS exposure increased nuclear factor erythroid-derived 2-like 2 (Nrf2) expressions by cardiac myocytes and endothelial cells, and these effects were potentiated by the combination of GA and WPS. WPS also increased DNA damage and cleaved caspase 3, and GA administration prevented these effects. Our data, obtained in experimental murine model of WPS exposure, show that GA ameliorates WPS-induced coagulation and cardiovascular inflammation, oxidative stress, DNA damage and apoptosis, through a mechanism involving Nrf2 activation." As taken from Nemmar A et al. 2019. Front. Physiol. 10, 53. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30858803

"In this study we investigated the hepatoprotective effects and possible mechanism of Acacia catechu in acetaminophen (APAP) induced hepatotoxicity using female Wistar rat model. Hepatotoxicity was induced by oral administration of acetaminophen (750 mg/kg body weight) for 24 h. The seed (400 mg/kg body weight) and bark (400 mg/kg body weight) extract's treated groups exhibited hepatoprotective effects and was compared with well-known clinical anti-dote N-acetylcysteine (NAC). When groups treated with acetaminophen, significant increase of liver weight/body weight ratio, liver function enzymes such as alanine aminotransferase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST) and decrease of antioxidant enzymes such as glutathione (GSH) and superoxide dismutase (SOD) were observed. The histopathology of APAP treated groups also showed moderate degree of sinusoidal congestion, centrilobular necrosis with polymorph nuclear cells infiltration, marked vacuolations and congestion. However, pretreatment with seed or bark extract groups decreased LPO accumulation, reduced the liver function enzymes and increased antioxidant defense enzymes. Moreover, histopathology of seed extract treated groups showed normal architecture whereas bark extract treated groups exhibited mild degree of vacuolations in the hepatocytes with minimal sinusoidal congestion. Taken together, our study concludes that A. catechu seed extract to be a more promising agent for protecting liver from APAP induced hepatotoxicity." As taken from Lakshmi T et al. 2018. Biomed. Pharmacother. 108, 838-844. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30372895

5. Toxicity

5.1. Single dose toxicity

Organism	Test Type	Route	Reported Dose	Effect	Source

hamster	LD50	oral	> 18gm/kg		Food & Drug Research Laboratories, Inc., Papers. Vol. 124, Pg. -, 1976.
mouse	LD50	oral	> 16gm/kg		Food & Drug Research Laboratories, Inc., Papers. Vol. 124, Pg. -, 1976.
rabbit	LD50	oral	8gm/kg		Food & Drug Research Laboratories, Inc., Papers. Vol. 124, Pg. -, 1976.
rat	LD50	oral	> 16gm/kg		Food & Drug Research Laboratories, Inc., Papers. Vol. 124, Pg. -, 1976.

As taken from RTECS, 2013

Reported Fatal Dose:

1. 1= Practically Non-Toxic: Probable Oral Lethal Dose (Human) Above 15 G/Kg, More Than 1 Quart For 70 Kg Person (150 LB). [Gosselin, R.E., H.C. Hodge, R.P. Smith, and M.N. Gleason Clinical Toxicology of Commercial Products 4th ed. Baltimore: Williams and Wilkins, 1976. p. II-155] **PEER REVIEWED**

As taken from HSDB, 2002.

Background: Acacia senegal is a plant traditionally used for its various properties, including the treatment of infectious diseases. Recently, our team has demonstrated the ability of the hydroethanolic extract of the leaves to increase the activity of phenicol antibiotics against multi-resistant bacteria. The aim of this work is to determine the toxicological effects of the extract and its capacity to inhibit the bacterial mobility of Gram-negative bacteria, in order to evaluate the level of safety use of this plant.

Methods: The cytotoxicity test was performed using the neutral red absorption method. Acute and sub-acute oral toxicity were conducted on NMRI mice and Wistar rats. The behaviour and adverse effects were recorded during the 14 days of the acute study. For the subacute test, biochemical parameters, food and water consumption, and morphological parameters were determined. The anti-motility activities were evaluated on Pseudomonas aeruginosa PA01 and Escherichia coli AG100, using specific concentrations of Agar as required by the method.

Results: HEASG induced inhibition of keratinocytes cell growth with an IC_{50} of 1302 ± 60 µg/mL. For the acute toxicity study in mice, the single dose of extract of 2000 mg/kg body weight caused no deaths and no behavioural changes were observed; therefore, the median lethal dose (LD_{50}) of HEASG was calculated to 5000 mg/kg body weight. In Wistar rats, no mortality was observed at 250, 500 and 1000 mg/kg/day during the 28-day subacute oral toxicity study. The weights of both females and males increased globally over time, regardless of the batch. No statistically significant differences were registered for organ weights and biochemical parameters, except for chloride for biochemical parameters. Water and food consumption did not change significantly. Furthermore, no macroscopic changes in organ appearance were observed. Regarding anti-motility activity, the extract has reduced the swarming motility of PA01 and AG100 significantly at the concentration of 32 µg/mL ($P < 0.001$). The extract has reduced the swimming motility ($P < 0.01$) of PA01 but not AG100.

Conclusions: The results suggest that hydroethanolic extract of A. senegal leaves has significantactivity against bacterial motility and relatively low toxicity.As taken from Magnini RD et al. BMC Complement Med Ther 2021. Available at https://pubmed.ncbi.nlm.nih.gov/34187452/

5.2. Repeated dose toxicity

Repeated Oral Admin Of Gum Arabic To Rats Caused Uncoupling Of Oxidative Phosphorylation In Liver & Heart Mitochondria & Partial Inhibition Of Mixed Function Oxidases Of Liver Endoplasmic

Reticulum. [BACHMANN E ET AL; PHARMACOLOGY (BASEL) 17 (1): 39 (1978)] **PEER REVIEWED**

As taken from HSDB, 2002.

Short-term studies

“Rat. Groups of rats were fed 0 per cent. or 15 per cent. arabic gum in their diet for 62 days. A cathartic effect was observed but weight gain, food efficiency, haematological findings and organ weights were normal (Booth et al., 1963). Guinea-pig Groups of 10 and 20 guinea-pigs were fed 15 per cent. Powdered arabic gum for six weeks. Controls received no bulk food in their diet. Weight gain was improved in the test groups (Booth et al., 1949). Rabbit A group of four rabbits was given 20 per cent. arabic gum in a casein diet for four weeks. Weight gain improved significantly in the test groups (Hove & Herndon, 1957). Dog Three dogs were given 32-35 intravenous injections of acacia over a period of 76 days at a total cumulative dosage ranging from 15.7-47.7 g/kg. The dog on the largest dose died with an enlarged liver but unexplained cause of death four months after its last injection. The other two dogs remained in good condition; biopsy showed acacia present in their livers 26 months after their last injections (Smalley et al., 1945). Man. Nine patients with nephrotic edema received one to six intravenous injections of acacia over periods up to eight weeks, with total doses ranging from 80-325 g. There were no signs or symptoms of liver enlargement, and no other complications. Five of these patients excreted in the urine 5.5 per cent. to 38 per cent. of a single dose during periods ranging from 10-30 days respectively (Johnson & Newman, 1945).”

As taken from JECFA, 1982.

This study was designed to evaluate and characterize any subchronic toxicity of a new type of gum arabic (SUPER GUM™ [Acacia(sen)SUPER GUM™]), a naturally processed polysaccharide exudate from gum acacia trees (Acacia senegal), when administered to both sexes of F344 rats at dietary levels of 0 (control), 1.25%, 2.5%, and 5.0% (10 rats/sex/group). During the study, the treatment had no effects on clinical signs, survival, body weights, and food and water consumption, or on findings of urinalysis, ophthalmology, hematology, or blood biochemistry. Gross pathology and histopathology exhibited no differences of toxicological significance between control and treated rats. Increased relative cecum (filled) weights, evident in both sexes of 5.0% group and females of 1.25% and 2.5% groups, were considered to be a physiological adaptation. Thus, the results indicated the toxic level of SUPER GUM™ to be more than 5.0%, and the no observed adverse effect level (NOAEL) was concluded to be 5.0% (3117 mg/kg body weights/day for males, and 3296 mg/kg body weights/day for males) from the present study. (Doi Y. et al., 2006)

This study evaluated the cardiovascular and renal effects of dietary fibre supplementation with Acacia (sen) SUPERGUM™ (Gum Arabic) in normal individuals and a group of diabetic nephropaths. The normal diet was supplemented with 25 g of SUPERGUM™ daily for a period of 8–12 weeks. For the whole cohort dietary supplementation with SUPERGUM™ resulted in a fall in mean systolic blood pressure [SBP] (138.4 ± 18.9 mmHg to 132.83 ± 15.9 mmHg p = 0.01). Of note was a significant fall in SBP seen in normal individuals who neither had hypertension nor diabetes (129.1 ± 8.3 mmHg vs 123.6 ± 11.5 mmHg, n = 10 p = 0.02). Parameters of arterial stiffness were examined in patients with diabetic nephropathy and a fall in MAP. In this subgroup there was a significant fall in both central systolic and diastolic blood pressures, with no alterations in AI, AI @75 or PWV. This suggests that the beneficial effects of SUPERGUM on blood pressure are not the result of alterations in arterial stiffness. There were no effects of SUPERGUM on renal function and hemodynamics in patients with diabetic nephropathy. In contrast a reversible change in GFR (113.0 ml min vs. 99.4 ml/min, p = 0.02) and ERPF (489.7 ml/min vs 463.0 ml/min, p = 0.04) was shown in the population of healthy volunteers. The key finding of this study is the a significant beneficial effect of dietary supplementation with SUPERGUM™ on blood pressure which is seen in both a patient group with diabetes and mild renal involvement as well as in a normal healthy normotensive cohort. (Glover D.A. et al., 2009)

Anderson et al. (1984) fed three groups of three male Albino Wistar rats (weights = 140 to 160 g) diets containing 1%, 4%, and 8% (w/w) gum arabic (Acacia Senegal Gum), respectively,daily for 28 days. A fourth group served as the negative control. At necropsy, hepatic and cardiac tissues were obtained for electron microscopy and microsomal P-450 assays. No discernible ultrastructural differences were observed between the livers of test (all dietary groups) and control rats; particularly, the mitochondria were normal. Also, no discernible ultrastructural differences were found between the hearts of test (all dietary groups) and control rats. Particularly, both the appearance and concentration of the mitochondria and myofibrils were identical in this comparison. The results of assays of hepatic microsomal protein and cytochrome P-450 for each dietary group indicated that gum arabic did not cause inductive effects. The investigators noted that when induction by active agents (e.g., phenobarbitone) takes place, cytochrome P-450 values are increased by several-fold within a few days (Anderson et al. 1984). Anderson et al. (1986) fed 10% (w/w) Gum Arabic (Acacia Senegal Gum) daily for 45 days to Wistar albino rats (99 to 120 g). The number of rats in the study was not stated. The rats were then killed by cervical dislocation while under ether anesthesia. Portions of the jejunum, ileum, and cecum were excised and the ultrastructure of each was evaluated using transmission electron microscopy. No abnormalities in organelles were observed within cells of the jejunum, ileum, or cecum of rats fed gum arabic. Additionally, neither inclusions nor other pathological changes were detected. It was concluded that no significant ultrastructural differences occurred between experimental and control rats (Anderson et al. 1986)".

As taken CIR, 2005.

Anderson et al. (1982) evaluated the subchronic oral toxicity of gum arabic (Acacia Senegal Gum) in two experiments using albino Wistar rats (24 to 28 days old). Body weights prior to initiation of the study were not included. In the first experiment, groups of 15 male rats were fed gum arabic at concentrations of 0.91% (dietary level = 0.53 g/ kg/day), 2.0% (1.08 g/kg/day), 4.3% (2.55 g/kg/day), and 8.6% (5.22 g/kg/day), respectively, for 13 weeks. Groups of 15 female rats were fed concentrations of 0.75% (0.5 g/kg/day), 1.7% (1.05 g/ kg/day), 3.7% (2.6 g/kg/day), and 7.5% (5.31/g/kg/day), respectively. Fifteen males and 15 females served as controls. In the second experiment, 15 male rats were fed gum arabic at an average concentration of 18.6% (14 g/kg/day) for 13 weeks. Fifteen female rats were fed an average concentration of 18.1% (13.8 g/kg/day). The two control groups consisted of 15 males and 15 females, respectively. Urine and blood samples were obtained during the study. The animals were killed under anesthesia by cervical dislocation at the end of the treatment period and prepared for necropsy. The results for the two experiments included the reported deaths of two control female rats. Growth rates were not reduced for male or female rats at dietary doses up to 5 g/kg/day (~8.5% gum arabic in diet). At a concentration of approximately 18% in the diet (14 g/kg/day), male rats had a reduced growth rate and smaller final body weight ($p < .01$). The average weight gain for male rats was 78% of that of controls. Following the ingestion of gum arabic, 5 g/kg/day, by male rats, kidney weights (absolute and relative to body weight) were reduced ($p < .05$). At the highest dietary doses tested (~18%, 14 g/kg/day), kidney weights for male and female rats were significantly reduced ($p < .01$). Liver weight was reduced in a dose-dependent manner in male rats; the difference between experimental and control groups was not significant at doses of gum arabic less than 5 g/kg/day. No significant differences were observed in urine volume or composition between control and test groups at any of the dietary concentrations of gum arabic tested. Similarly, no significant hematological changes were observed between test and control groups. At microscopic examination, no alterations were found that were attributable to the ingestion of gum arabic. The only treatment-related alteration noted at necropsy was Cecal enlargement in rats of the highest dose groups (Anderson et al. 1982). In another study, Anderson et al. (1984) fed four groups of five male albino Wistar rats (weights = 40 to 60 g) diets containing 0.5%, 1.5%, 2.5%, and 3.5% (w/w) Gum Arabic (Acacia Senegal Gum), respectively, daily for 91 days. A fifth group served as the negative control. At the end of the feeding period, the animals were killed by cervical dislocation for necropsy. Samples of liver and heart from each treatment group were obtained for transmission electron microscopy. Livers from the remaining rats

(two per group) were used for assays of microsomal protein and cytochrome P-450. Electron microscopic findings for cardiac muscle included no abnormality of myofilaments, no depletion of glycogen reserves, no abnormality of the intra-cytoplasmic mitochondria or endoplasmic reticulum, no excessive infiltration with lipid, and no evidence of interstitial infiltration. Additionally, no abnormalities were observed with respect to the size, chromatin content, or nucleoli of nuclei. Electron microscopic findings for the liver included no abnormalities in hepatocytes, Kupffer cells, or lining cells of the biliary passages. The mitochondria and nuclei were normal both in appearance and internal structure, and no abnormalities were observed in intra-cytoplasmic glycogen stores (Anderson et al. 1984)"

As taken from CIR, 2005.

"Five healthy male subjects (30 to 55 years old) ingested 25g gum arabic (Acacia Senegal Gum) daily for 21 days. Toxic effects were not observed during the 21-day period; breath hydrogen concentrations increased only after chronic administration. The fact that gum arabic was not recovered from the feces suggest that it is degraded extensively in the human colon (Anderson 1986)."

OBSERVATIONS IN MAN

"Nine patients with nephrotic edema received one to six intravenous injections of acacia over periods up to eight weeks, with total doses ranging from 80-325 g. There were no signs or symptoms of liver enlargement, and no other complications. Five of these patients excreted in the urine 5.5% to 38% of a single dose during periods ranging from 10 to 30 days, respectively (Johnson & Newman, 1945)."

"Acacia gum (E414) (EFSA-Q-2011-00513): application of the decision tree on the risk assessment of food additives with "no numerical ADI and generally authorized at QS use":

…. Further to sub-chronic studies in rodents a NOAEL of 5000 mg/kg bw/day has been identified and used as a point of departure (POD). Applying the appropriate UF an ADI of 25 mg/kg bw/day could be derived. However in case of food additives characterized by "low intrinsic toxicity" and are authorized at QS, the decision to allocate an ADI should be further discussed."

As taken from EFSA, 2014.

Type of Test	Route of Exposure	Species Observed	Dose Data	Toxic Effects	Reference
TDLo - Lowest published toxic dose	Oral	Rodent - rat	1260 gm/kg/13W (continuous)	Liver - changes in liver weight Kidney/Ureter/Bladder - changes in bladder weight Blood - changes in serum composition (e.g. TP, bilirubin, cholesterol)	TOLED5 Toxicology Letters. (Elsevier Science Pub. B.V., POB 211, 1000 AE Amsterdam, Netherlands) V.1- 1977- Volume(issue)/page/year: 14,221,1982

As taken from RTECS, 2013.

"The subchronic (13 weeks) oral toxicity of acacia gum was investigated by Anderson et al. (1982). The animals received acacia gum in their diet and the study was conducted in two consecutive experimental phases. In the first one, the rats were given doses ranging from 0 to about 5,000 mg acacia gum/kg body weight (bw) per day, and in the second phase, they received 0 or 14,000 mg acacia gum/kg bw per day. The Panel noted that these two studies were done independently and that merging their data may not be straightforward. The Panel considered that no toxicological effect was observed in these studies by Anderson et al. (1982). From the first study, no adverse effects have been identified up to 5,220 and 5,310 mg acacia gum/kg bw per day in male and

female, respectively, the highest dose tested. Overall, the short-term and subchronic administration of oral doses up to 5,000 mg acacia gum/kg bw per day to rats and 20,000 mg acacia gum/kg bw per day to mice, the highest doses tested, did not induce any biologically relevant adverse effects. In some studies, caecal enlargement was observed. The Panel considered that an increased caecum weight in animals fed high amounts of carbohydrates is considered as a physiological response to an increased fermentation by the intestinal microbiota."

As taken from EFSA, 2017

"Three human studies on gum arabic found that daily ingestion by adults of up to 30 g (equivalent to 500 mg/kg bw per day for a 60 kg individual) over 18–21 days was well tolerated (Ross et al., 1983; Sharma, 1985; Cherbut et al., 2003)."

As taken from JECFA, 2019.

Short-term toxicity• 14-Day (Dosed-Feed) (C50748) Completed

- Rats: F344/N; Mice: B6C3F1
- 13-Week (Dosed-Feed) (C50748) Completed
- Rats: F344/N; Mice: B6C3F1
- Dose: 0, 6300, 100000 PPM/10 PER GROUP.

As taken from NTP, 2022

5.3. Reproduction toxicity

"Groups of 21-24 pregnant Wistar-derived rats were dosed by gavage on days 6 through 15 of gestation with 0, 16, 75, 350 or 1600 mg/kg of arabic gum suspended in corn oil. No compound-related effect was observed on nidation, maternal or foetal survival, or on the incidence of hard or soft tissue anomalies occurring in the offspring. The average foetal weight at birth was slightly depressed in the high-dose group. Groups of 19-21 pregnant CD-1 mice were dosed by gavage on days 6 through 15 of gestation with 0, 16, 75, 350 or 1600 mg/kg of arabic gum suspended in corn oil. No compound-related effect was observed on nidation, maternal or foetal survival or on the incidence of hard or soft tissue anomalies occurring in the offspring. The average foetal weight at birth was slightly depressed in the high-dose group. Groups of 19-21 pregnant outbred golden hamsters were dosed by gavage on days 6 through 10 of gestation with 0, 16, 75, 350 or 1600 mg/kg of arabic gum suspended in corn oil. No compound-related effect was observed on nidation, maternal or foetal survival or on the incidence of hard or soft tissue anomalies occurring in the offspring. Groups of 12-14 pregnant Dutch-belted rabbits were dosed by gavage with 0, 8, 37, 173 or 800 mg/kg of arabic gum suspended in corn oil on days 6 through 18 of gestation. The administration of up to 37 mg/kg of the test material as a suspension in anhydrous corn oil had no clear effect on nidation or on maternal or foetal survival. The number and type of abnormalities seen in foetal soft or skeletal tissues derived from this group of does did not differ from the number occurring spontaneously in the sham-treated controls. However, in 2 groups of dams dosed at 173 and 800 mg/kg bw respectively, maternal toxicity ensued with the loss of a majority of animals in the 800 mg/kg group. Death was preceded by severe bloody diarrhoea, urinary incontinence, with anorexia for 48-72 hours terminally. At autopsy no gross pathological findings were seen other than haemorrhage in the mucosa of the small intestines. Does which survived the highest dose and bore living young to term remained outwardly normal, and the offspring were likewise normal in all respects. It was concluded that this test substance was not a teratogen in the rabbit under the test conditions employed (Morgareidge, 1972)."

As taken from JECFA, 1982.

Gum arabic in the diet at 0, 1, 2, 4, 7.5 or 15% was available ad lib. to male and female Osborne-Mendel rats during premating and mating and throughout gestation. During gestation, the treated

females consumed from 683 mg gum/kg body weight/day in the 1% group to 10,647 mg gum/kg/day in the 15% group. The animals were killed on gestation day 20. There were no dose-related changes in maternal findings, number of foetuses, foetal viability or external, visceral or skeletal variations. No terata were seen. (Collins T.F.X. et al., 1987).

Type of Test	Route of Exposure	Species Observed	Dose Data	Sex/Duration	Toxic Effects	Reference
TDLo - Lowest published toxic dose	Oral	Rodent - rat	350 gm/kg	male 10 week(s) pre-mating	Reproductive - Fertility - pre-implantation mortality (e.g. reduction in number of implants per female; total number of implants per corpora lutea)	ENMUDM Environmental Mutagenesis. (New York, NY) V.1-9, 1979-87. For publisher information, see EMMUEG. Volume(issue)/page/year: 8,357,1986

As taken from RTECS, 2013.

“ In a dietary combined fertility and developmental toxicity study in rats (Collins et al., 1987), a no observed adverse effect level (NOAEL) of 10,647 mg acacia gum/kg bw per day for reproductive, developmental and parental effects was identified, the highest dose tested. In addition, other reproductive studies in rats showed no effects at the highest dose tested (Morseth and Ihara (1989a), Huynh et al., 2000). In the identically performed prenatal developmental tests with acacia gum by gavage in mice, rats and hamsters (FDRL, 1972b), 1,600 mg/kg bw per day (the highest dose tested) showed no dose-related developmental effects.”

As taken from EFSA, 2017

“The effects of the perinatal oral exposure to Gum Arabic (GA) on mice offspring was examined. GA was added to the drinking water of pregnant female Swiss-Webster strain mice at doses of 1 and 4 g/kg body weight, starting from the first day of pregnancy. The treatment continued until the fifteenth day after delivery, after which mothers were switched to plain tap water. A number of tests were carried out on offspring starting one day after birth and extending up to postnatal day 30 (PD30). Pups showed a reduced gain of body weight and delayed opening of the eyes in comparison to the control group and only pups exposed to 1 g/kg body weight GA had a faster appearance of hair. Sensory motor reflex tests carried out during the weaning period (from day of birth to PD21) showed enhanced motor reflexes in pups exposed to GA. During the adolescent period (from PD22 to PD30), offspring showed dose-dependent enhanced motor activity (on PD22), reduced anxiety and fear (on PD27) and slightly enhanced memory and learning abilities (on PD30). Biochemical tests of a number of blood parameters were conducted during and after the weaning period (on PD15 and PD30, respectively). Our results indicated that GA might have a hypoglycemic and a beneficial effect on red and white blood cell counts. This study gives a first insight on the effect of GA consumption on offspring, providing a starting point for further studies.” As taken from Binjumah M et al. 2019. Saudi J. Biol. Sci. 25(7), 1332-1338. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30505178

“The current study has been designed to evaluate the impact of cisplatin on the spermatogenesis, histopathological pictures, DNA damage, and oxidative stress parameters in the testicular tissues for a long period of time. Additionally, we clarify the conservative effect of gum arabic against all of the above toxicological parameters. Twenty male Wister rats were used and divided into four equal groups (n = 5). Group 1 was used as a control negative group. Group 2 was received gum arabic (7.5 mg/kg bwt) daily in drinking water. Group 3 was administered with a single i.p. dose of cisplatin

(7 mg/kg bwt). Group 4 was received both cisplatin and gum arabic. Body and testis weights, epididymal sperm analysis, blood testosterone level, testis oxidative stress markers, histopathological pictures, and immunohistochemical staining of the testicular tissues were done. Our results showed that the cisplatin administration led to a significant reduction in the body and testis weights, sperm count, sperm motility, blood testosterone level, and testicular antioxidants. Additionally, it led to a significant elevation of sperm apoptosis, testicular malondialdehyde levels, DNA damage, histopathological alteration, and caspase-3 immunostaining reactions. On the other hand, pretreatment of rats with gum arabic was improved all of the previous parameters. From our findings, we can conclude that the gum arabic could be used as an antioxidant and anti-apoptotic agent to alleviate the toxicity of cisplatin on the testis." As taken from Azouz RA and Hassanen EI. 2020. Rev. Bras. Farmacogn. 30, 90-98. Available at https://link.springer.com/article/10.1007/s43450-020-00015-7

"SUMMARY: Diabetes mellitus is a serious disease with a high incidence of occurrence in our community. Gum Arabic (GA) is a branched-chain polysaccharide which has strong antioxidant properties, and has been used to reduce the experimental toxicity. Yet, the effects of GA on testicular tissue in type I diabetic rats have not been enough investigated. This study was designed to investigate histological changes in testes of male Wistar rats and investigate the protective potential of GA against diabetes- induced testicular toxicity in rats. Fifty adult male Wistar rats were assigned into five groups (n = 10 of each): Group 1 (non-diabetic rats) served as control, Group 2 served as diabetic group injected with Alloxan, Group 3 diabetic group plus insulin, Group 4 diabetic group given 15 % GA in drinking water and Group 5 diabetic group plus insulin and GA for 4 weeks. Compared to control group, histopathological examinations of testicular tissue from the diabetic rats group, showed degeneration, necrosis and atrophy of seminiferous with presence of giant cells. Necrosis and hemorrhage in the renal tissue. On the other hand, treatment with GA ameliorated all the previous histological changes. Overall, oral administration of GA alone or with insulin daily for 4 weeks successfully ameliorated the testicular histological changes. These data demonstrated that GA significantly improved diabetes complication in rat testis. This study suggested that GA might have a protective effect against oxidative stress-induced impaired testicular functions in diabetic rats. The possible mechanisms of this action might be ascribed to their antioxidant and anti-inflammatory properties." As taken from Al-Doaiss AA and Al-Shehri MA 2020. Int. J. Morphol., 38(2), 340-347. Available at https://bit.ly/2VMT6CC

"The Panel, performing a literature search, identified a publication on the effects of gum arabic in which development, behaviour and biochemical parameters were tested after administration via drinking water of 0, 1 and 4 mg/kg bw per day to female mice (Swiss-Webster strain) from gestation day (GD) 0 to postnatal day (PND) 15 (Binjumah et al., 2018). When reviewing the publication, the Panel noted that the study and the reporting showed several serious flaws and, therefore, considered that the study cannot be used for risk assessment."

As taken from EFSA, 2019.

"In this study the effect of Gum arabic (Acacia Senegal) was systemically targeted at male fertility with two experiments, the first comparing the effectiveness of Gum arabic (GA) and Tribulus terrestris (TT). For the first experiment, 27 adult mice Balb / c (18 females, 9 males) were divided into 3 in each group, one male and two females, group one had the usual tap water as power, group two had 5% (w / v) GA and group three had 5% (w / v) of TT for 21 days. The results showed, the number of offspring was more with GA treated when compared to TT treated. Blood measurements of testosterone showed significant increase in the GA group as compared to other groups, also Histopathological analysis showed the dose dependent 5% GA had normal seminiferous tubules with increase spermatogenesis. In this study the enhanced fertility in GA-treated mice Balb/c was observed and the experimental studies also show that GA fertility was increased." As taken from Nasir O et al. 2020. Saudi Pharm. J. 28(12), 1791-1796. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33424268/

5.4. Mutagenicity

“Gum arabic did not produce a measurable mutagenic response or alteration in the recombination frequency for Saccharomyces cerevisiae in either the host-mediated assay or in vitro. Similarly, no mutagenicity was reported with gum arabic either in the host-mediated assay or in vitro using Salmonella strains G-46 or TA-1530. Cultures of bone marrow metaphase chromosomes taken from rats dosed in vivo with 50 mg or 2.5 g/kg of arabic gum showed an increased incidence of chromosomal breaks occurring within 6 hours of treatment. Similar effects were found in vitro with human WI-38 embryonic lung cells. Gum arabic was tested using the dominant lethal gene test in Sprague-Dawley rats. Males were given 0, 30, 2500 or 5000 mg/kg by gavage in a water suspension. A significant increase in dead implants was noted in pregnant females mated to males given a single dose of 5000 mg/kg at the third week of the study. No other significant effects were recorded and arabic gum was considered not to be mutagen in this study (Newell & Maxwell, 1972). No genetic activity was noted in in vitro mutagenic tests with Saccharomyces cerevisiae strain D4 and Salmonella typhimurium strains TA-1535, TA-1537 and TA-1538. Both suspension and plate tests were used, with and without activation. Activation systems were prepared from liver, lung, kidney and testes from male mice, rats and monkeys (Brusick, 1975). Gum arabic was concluded to be not mutagenic based on the sex- linked dominant lethal test in Drosophila (Valencia & Abrahamson).”

As taken from JECFA, 1982.

Mutagenicity Studies:

Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA98
Metabolic Activation:	NONE
Method:	STANDARD PLATE
Dose:	0.033-10 MG/PLATE (TEST MATERIAL SOLVENT: POTASSIUM OR SODIUM PHOSPHATE BUFFER)
Results:	NEGATIVE
Reference:	[PRIVAL, MJ, SIMMON, VF AND MORTELMANS,KE; BACTERIAL MUTAGENICITY TESTING OF 49 FOOD INGREDIENTS GIVES VERY FEW POSITIVE RESULTS; MUTAT. RES. 260(4):321-329, 1991]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA100
Metabolic Activation:	NONE
Method:	STANDARD PLATE
Dose:	0.033-10 MG/PLATE (TEST MATERIAL SOLVENT: POTASSIUM OR SODIUM PHOSPHATE BUFFER)
Results:	NEGATIVE

Reference:	[PRIVAL,MJ, SIMMON,VF AND MORTELMANS,KE; BACTERIAL MUTAGENICITY TESTING OF 49 FOOD INGREDIENTS GIVES VERY FEW POSITIVE RESULTS; MUTAT. RES. 260(4):321-329, 1991]

Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA1535
Metabolic Activation:	NONE
Method:	STANDARD PLATE
Dose:	0.033-10 MG/PLATE (TEST MATERIAL SOLVENT: POTASSIUM OR SODIUM PHOSPHATE BUFFER)
Results:	NEGATIVE
Reference:	[PRIVAL,MJ, SIMMON,VF AND MORTELMANS,KE; BACTERIAL MUTAGENICITY TESTING OF 49 FOOD INGREDIENTS GIVES VERY FEW POSITIVE RESULTS; MUTAT. RES. 260(4):321-329, 1991]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA1537
Metabolic Activation:	NONE
Method:	STANDARD PLATE
Dose:	0.033-10 MG/PLATE (TEST MATERIAL SOLVENT: POTASSIUM OR SODIUM PHOSPHATE BUFFER)
Results:	NEGATIVE
Reference:	[PRIVAL, MJ, SIMMON, VF AND MORTELMANS,KE; BACTERIAL MUTAGENICITY TESTING OF 49 FOOD INGREDIENTS GIVES VERY FEW POSITIVE RESULTS; MUTAT. RES. 260(4):321-329, 1991]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA1538
Metabolic Activation:	NONE
Method:	STANDARD PLATE
Dose:	0.033-10 MG/PLATE (TEST MATERIAL SOLVENT: POTASSIUM OR SODIUM PHOSPHATE BUFFER)
Results:	NEGATIVE
Reference:	[PRIVAL,MJ, SIMMON,VF AND MORTELMANS,KE; BACTERIAL MUTAGENICITY TESTING OF 49 FOOD INGREDIENTS GIVES VERY FEW POSITIVE RESULTS; MUTAT. RES. 260(4):321-329, 1991]

Test System:	AMES SALMONELLA TYPHIMURIUM	
Strain Indicator:	TA98	
Metabolic Activation:	RAT, LIVER, S-9, AROCLOR 1254	
Method:	STANDARD PLATE	
Dose:	0.033-10 MG/PLATE (TEST MATERIAL SOLVENT: POTASSIUM OR SODIUM PHOSPHATE BUFFER)	
Results:	NEGATIVE	
Reference:	[PRIVAL,MJ, SIMMON,VF AND MORTELMANS,KE; BACTERIAL MUTAGENICITY TESTING OF 49 FOOD INGREDIENTS GIVES VERY FEW POSITIVE RESULTS; MUTAT. RES. 260(4):321-329, 1991]	
Test System:	AMES SALMONELLA TYPHIMURIUM	
Strain Indicator:	TA100	
Metabolic Activation:	RAT, LIVER, S-9, AROCLOR 1254	
Method:	STANDARD PLATE	
Dose:	0.033-10 MG/PLATE (TEST MATERIAL SOLVENT: POTASSIUM OR SODIUM PHOSPHATE BUFFER)	
Results:	NEGATIVE	
Reference:	[PRIVAL,MJ, SIMMON,VF AND MORTELMANS,KE; BACTERIAL MUTAGENICITY TESTING OF 49 FOOD INGREDIENTS GIVES VERY FEW POSITIVE RESULTS; MUTAT. RES. 260(4):321-329, 1991]	
Test System:	AMES SALMONELLA TYPHIMURIUM	
Strain Indicator:	TA1535	
Metabolic Activation:	RAT, LIVER, S-9, AROCLOR 1254	
Method:	STANDARD PLATE	
Dose:	0.033-10 MG/PLATE (TEST MATERIAL SOLVENT: POTASSIUM OR SODIUM PHOSPHATE BUFFER)	
Results:	NEGATIVE	
Reference:	[PRIVAL,MJ, SIMMON,VF AND MORTELMANS,KE; BACTERIAL MUTAGENICITY TESTING OF 49 FOOD INGREDIENTS GIVES VERY FEW POSITIVE RESULTS; MUTAT. RES. 260(4):321-329, 1991]	
Test System:	AMES SALMONELLA TYPHIMURIUM	
Strain Indicator:	TA1537	

Metabolic Activation:	RAT, LIVER, S-9, AROCLOR 1254	
Method:	STANDARD PLATE	
Dose:	0.033-10 MG/PLATE (TEST MATERIAL SOLVENT: POTASSIUM OR SODIUM PHOSPHATE BUFFER)	
Results:	NEGATIVE	
Reference:	[PRIVAL,MJ, SIMMON,VF AND MORTELMANS,KE; BACTERIAL MUTAGENICITY TESTING OF 49 FOOD INGREDIENTS GIVES VERY FEW POSITIVE RESULTS; MUTAT. RES. 260(4):321-329, 1991]	
Test System:	AMES SALMONELLA TYPHIMURIUM	
Strain Indicator:	TA1538	
Metabolic Activation:	RAT, LIVER, S-9, AROCLOR 1254	
Method:	STANDARD PLATE	
Dose:	0.033-10 MG/PLATE (TEST MATERIAL SOLVENT: POTASSIUM OR SODIUM PHOSPHATE BUFFER)	
Results:	NEGATIVE	
Reference:	[PRIVAL,MJ, SIMMON,VF AND MORTELMANS,KE; BACTERIAL MUTAGENICITY TESTING OF 49 FOOD INGREDIENTS GIVES VERY FEW POSITIVE RESULTS; MUTAT. RES. 260(4):321-329, 1991]	
Test System:	E. COLI	
Strain Indicator:	WP2	
Metabolic Activation:	NONE	
Method:	STANDARD PLATE	
Dose:	0.033-10 MG/PLATE (TEST MATERIAL SOLVENT: POTASSIUM OR SODIUM PHOSPHATE BUFFER)	
Results:	NEGATIVE	
Reference:	[PRIVAL,MJ, SIMMON,VF AND MORTELMANS,KE; BACTERIAL MUTAGENICITY TESTING OF 49 FOOD INGREDIENTS GIVES VERY FEW POSITIVE RESULTS; MUTAT. RES. 260(4):321-329, 1991]	
Test System:	E. COLI	
Strain Indicator:	WP2	
Metabolic Activation:	RAT, LIVER, S-9, AROCLOR 1254	
Method:	STANDARD PLATE	

Dose:	0.033-10 MG/PLATE (TEST MATERIAL SOLVENT: POTASSIUM OR SODIUM PHOSPHATE BUFFER)	
Results:	NEGATIVE	
Reference:	[PRIVAL,MJ, SIMMON,VF AND MORTELMANS,KE; BACTERIAL MUTAGENICITY TESTING OF 49 FOOD INGREDIENTS GIVES VERY FEW POSITIVE RESULTS; MUTAT. RES. 260(4):321-329, 1991]	
Test System:	AMES SALMONELLA TYPHIMURIUM	
Strain Indicator:	TA100	
Metabolic Activation:	NONE	
Method:	PREINCUBATION	
Dose:	100-10000 UG/PLATE (TEST MATERIAL SOLVENT: DISTILLED WATER)	
Results:	NEGATIVE	
Reference:	[ZEIGER,E, ANDERSON,B, HAWORTH,S, LAWLOR,T AND MORTELMANS,K; SALMONELLA MUTAGENICITY TESTS. V. RESULTS FROM THE TESTING OF 311 CHEMICALS; ENVIRON. MOL. MUTAGEN. 19(SUPPL.21):2-141, 1992]	
Test System:	AMES SALMONELLA TYPHIMURIUM	
Strain Indicator:	TA100	
Metabolic Activation:	HAMSTER, LIVER, S-9, AROCLOR 1254 (10 OR 30%)	
Method:	PREINCUBATION	
Dose:	100-10000 UG/PLATE (TEST MATERIAL SOLVENT: DISTILLED WATER)	
Results:	NEGATIVE	
Reference:	[ZEIGER,E, ANDERSON,B, HAWORTH,S, LAWLOR,T AND MORTELMANS,K; SALMONELLA MUTAGENICITY TESTS. V. RESULTS FROM THE TESTING OF 311 CHEMICALS; ENVIRON. MOL. MUTAGEN. 19(SUPPL.21):2-141, 1992]	
Test System:	AMES SALMONELLA TYPHIMURIUM	
Strain Indicator:	TA100	
Metabolic Activation:	RAT, LIVER, S-9, AROCLOR 1254 (10 OR 30%)	
Method:	PREINCUBATION	
Dose:	100-10000 UG/PLATE (TEST MATERIAL SOLVENT: DISTILLED WATER)	
Results:	NEGATIVE	
Reference:	[ZEIGER,E, ANDERSON,B, HAWORTH,S, LAWLOR,T AND MORTELMANS,K; SALMONELLA MUTAGENICITY TESTS. V. RESULTS FROM THE TESTING OF 311	

	CHEMICALS; ENVIRON. MOL. MUTAGEN. 19(SUPPL.21):2-141, 1992]	
Test System:	AMES SALMONELLA TYPHIMURIUM	
Strain Indicator:	TA1535	
Metabolic Activation:	NONE	
Method:	PREINCUBATION	
Dose:	100-10000 UG/PLATE (TEST MATERIAL SOLVENT: DISTILLED WATER)	
Results:	NEGATIVE	
Reference:	[ZEIGER,E, ANDERSON,B, HAWORTH,S, LAWLOR,T AND MORTELMANS,K; SALMONELLA MUTAGENICITY TESTS. V. RESULTS FROM THE TESTING OF 311 CHEMICALS; ENVIRON. MOL. MUTAGEN. 19(SUPPL.21):2-141, 1992]	
Test System:	AMES SALMONELLA TYPHIMURIUM	
Strain Indicator:	TA1535	
Metabolic Activation:	HAMSTER, LIVER, S-9, AROCLOR 1254 (10 OR 30%)	
Method:	PREINCUBATION	
Dose:	100-10000 UG/PLATE (TEST MATERIAL SOLVENT: DISTILLED WATER)	
Results:	NEGATIVE	
Reference:	[ZEIGER,E, ANDERSON,B, HAWORTH,S, LAWLOR,T AND MORTELMANS,K; SALMONELLA MUTAGENICITY TESTS. V. RESULTS FROM THE TESTING OF 311 CHEMICALS; ENVIRON. MOL. MUTAGEN. 19(SUPPL.21):2-141, 1992]	
Test System:	AMES SALMONELLA TYPHIMURIUM	
Strain Indicator:	TA1535	
Metabolic Activation:	RAT, LIVER, S-9, AROCLOR 1254 (10 OR 30%)	
Method:	PREINCUBATION	
Dose:	100-10000 UG/PLATE (TEST MATERIAL SOLVENT: DISTILLED WATER)	
Results:	NEGATIVE	
Reference:	[ZEIGER,E, ANDERSON,B, HAWORTH,S, LAWLOR,T AND MORTELMANS,K; SALMONELLA MUTAGENICITY TESTS. V. RESULTS FROM THE TESTING OF 311 CHEMICALS; ENVIRON. MOL. MUTAGEN. 19(SUPPL.21):2-141, 1992]	
Test System:	AMES SALMONELLA TYPHIMURIUM	
Strain Indicator:	TA97	
Metabolic Activation:	NONE	

Method:	PREINCUBATION	
Dose:	100-10000 UG/PLATE (TEST MATERIAL SOLVENT: DISTILLED WATER)	
Results:	NEGATIVE	
Reference:	[ZEIGER,E, ANDERSON,B, HAWORTH,S, LAWLOR,T AND MORTELMANS,K; SALMONELLA MUTAGENICITY TESTS. V. RESULTS FROM THE TESTING OF 311 CHEMICALS; ENVIRON. MOL. MUTAGEN. 19(SUPPL.21):2-141, 1992]	
Test System:	AMES SALMONELLA TYPHIMURIUM	
Strain Indicator:	TA97	
Metabolic Activation:	HAMSTER, LIVER, S-9, AROCLOR 1254 (10 OR 30%)	
Method:	PREINCUBATION	
Dose:	100-10000 UG/PLATE (TEST MATERIAL SOLVENT: DISTILLED WATER)	
Results:	NEGATIVE	
Reference:	[ZEIGER,E, ANDERSON,B, HAWORTH,S, LAWLOR,T AND MORTELMANS,K; SALMONELLA MUTAGENICITY TESTS. V. RESULTS FROM THE TESTING OF 311 CHEMICALS; ENVIRON. MOL. MUTAGEN. 19(SUPPL.21):2-141, 1992]	
Test System:	AMES SALMONELLA TYPHIMURIUM	
Strain Indicator:	TA97	
Metabolic Activation:	RAT, LIVER, S-9, AROCLOR 1254 (10 OR 30%)	
Method:	PREINCUBATION	
Dose:	100-10000 UG/PLATE (TEST MATERIAL SOLVENT: DISTILLED WATER)	
Results:	NEGATIVE	
Reference:	[ZEIGER,E, ANDERSON,B, HAWORTH,S, LAWLOR,T AND MORTELMANS,K; SALMONELLA MUTAGENICITY TESTS. V. RESULTS FROM THE TESTING OF 311 CHEMICALS; ENVIRON. MOL. MUTAGEN. 19(SUPPL.21):2-141, 1992]	
Test System:	AMES SALMONELLA TYPHIMURIUM	
Strain Indicator:	TA98	
Metabolic Activation:	NONE	
Method:	PREINCUBATION	
Dose:	100-10000 UG/PLATE (TEST MATERIAL SOLVENT: DISTILLED WATER)	
Results:	NEGATIVE	
Reference:	[ZEIGER,E, ANDERSON,B, HAWORTH,S, LAWLOR,T AND MORTELMANS,K; SALMONELLA MUTAGENICITY TESTS. V. RESULTS FROM THE TESTING OF 311	

	CHEMICALS; ENVIRON. MOL. MUTAGEN. 19(SUPPL.21):2-141, 1992]	
Test System:	AMES SALMONELLA TYPHIMURIUM	
Strain Indicator:	TA98	
Metabolic Activation:	HAMSTER, LIVER, S-9, AROCLOR 1254 (10 OR 30%)	
Method:	PREINCUBATION	
Dose:	100-10000 UG/PLATE (TEST MATERIAL SOLVENT: DISTILLED WATER)	
Results:	NEGATIVE	
Reference:	[ZEIGER,E, ANDERSON,B, HAWORTH,S, LAWLOR,T AND MORTELMANS,K; SALMONELLA MUTAGENICITY TESTS. V. RESULTS FROM THE TESTING OF 311 CHEMICALS; ENVIRON. MOL. MUTAGEN. 19(SUPPL.21):2-141, 1992]	
Test System:	AMES SALMONELLA TYPHIMURIUM	
Strain Indicator:	TA98	
Metabolic Activation:	RAT, LIVER, S-9, AROCLOR 1254 (10 OR 30%)	
Method:	PREINCUBATION	
Dose:	100-10000 UG/PLATE (TEST MATERIAL SOLVENT: DISTILLED WATER)	
Results:	NEGATIVE	
Reference:	[ZEIGER,E, ANDERSON,B, HAWORTH,S, LAWLOR,T AND MORTELMANS,K; SALMONELLA MUTAGENICITY TESTS. V. RESULTS FROM THE TESTING OF 311 CHEMICALS; ENVIRON. MOL. MUTAGEN. 19(SUPPL.21):2-141, 1992]	
Test System:	AMES SALMONELLA TYPHIMURIUM	
Strain Indicator:	TA1537	
Metabolic Activation:	NONE	
Method:	PREINCUBATION	
Dose:	100-10000 UG/PLATE (TEST MATERIAL SOLVENT: DISTILLED WATER)	
Results:	NEGATIVE	
Reference:	[ZEIGER,E, ANDERSON,B, HAWORTH,S, LAWLOR,T AND MORTELMANS,K; SALMONELLA MUTAGENICITY TESTS. V. RESULTS FROM THE TESTING OF 311 CHEMICALS; ENVIRON. MOL. MUTAGEN. 19(SUPPL.21):2-141, 1992]	
Test System:	AMES SALMONELLA TYPHIMURIUM	
Strain Indicator:	TA1537	
Metabolic Activation:	HAMSTER, LIVER, S-9, AROCLOR 1254 (30%)	

Method:	PREINCUBATION	
Dose:	100-10000 UG/PLATE (TEST MATERIAL SOLVENT: DISTILLED WATER)	
Results:	NEGATIVE	
Reference:	[ZEIGER,E, ANDERSON,B, HAWORTH,S, LAWLOR,T AND MORTELMANS,K; SALMONELLA MUTAGENICITY TESTS. V. RESULTS FROM THE TESTING OF 311 CHEMICALS; ENVIRON. MOL. MUTAGEN. 19(SUPPL.21):2-141, 1992]	
Test System:	AMES SALMONELLA TYPHIMURIUM	
Strain Indicator:	TA1537	
Metabolic Activation:	RAT, LIVER, S-9, AROCLOR 1254 (30%)	
Method:	PREINCUBATION	
Dose:	100-10000 UG/PLATE (TEST MATERIAL SOLVENT: DISTILLED WATER)	
Results:	NEGATIVE	
Reference:	[ZEIGER,E, ANDERSON,B, HAWORTH,S, LAWLOR,T AND MORTELMANS,K; SALMONELLA MUTAGENICITY TESTS. V. RESULTS FROM THE TESTING OF 311 CHEMICALS; ENVIRON. MOL. MUTAGEN. 19(SUPPL.21):2-141, 1992]	
Test System:	AMES SALMONELLA TYPHIMURIUM	
Strain Indicator:	TA98	
Metabolic Activation:	NONE	
Method:	STANDARD PLATE	
Dose:	0-10 MG/ML (TEST MATERIAL SOLVENT: DMSO)	
Results:	NEGATIVE	
Reference:	[DE VEER,I, MORISKE,HJ AND RUEDEN,H; PHOTOCHEMICAL DECOMPOSITION OF ORGANIC COMPOUNDS IN WATER AFTER UV-IRRADIATION: INVESTIGATION OF POSITIVE MUTAGENIC EFFECTS; TOXICOL. LETT. 72(1-3):113-119, 1994]	
Test System:	AMES SALMONELLA TYPHIMURIUM	
Strain Indicator:	TA98	
Metabolic Activation:	RAT, LIVER, S-9, AROCLOR 1254	
Method:	STANDARD PLATE	
Dose:	0-10 MG/ML (TEST MATERIAL SOLVENT: DMSO)	
Results:	NEGATIVE	
Reference:	[DE VEER,I, MORISKE,HJ AND RUEDEN,H; PHOTOCHEMICAL DECOMPOSITION OF ORGANIC COMPOUNDS IN WATER AFTER UV-IRRADIATION: INVESTIGATION	

	OF POSITIVE MUTAGENIC EFFECTS; TOXICOL. LETT. 72(1-3):113-119, 1994]	
Test System:	AMES SALMONELLA TYPHIMURIUM	
Strain Indicator:	TA100	
Metabolic Activation:	NONE	
Method:	STANDARD PLATE	
Dose:	0-10 MG/ML (TEST MATERIAL SOLVENT: DMSO)	
Results:	NEGATIVE	
Reference:	[DE VEER,I, MORISKE,HJ AND RUEDEN,H; PHOTOCHEMICAL DECOMPOSITION OF ORGANIC COMPOUNDS IN WATER AFTER UV-IRRADIATION: INVESTIGATION OF POSITIVE MUTAGENIC EFFECTS; TOXICOL. LETT. 72(1-3):113-119, 1994]	
Test System:	AMES SALMONELLA TYPHIMURIUM	
Strain Indicator:	TA100	
Metabolic Activation:	RAT, LIVER, S-9, AROCLOR 1254	
Method:	STANDARD PLATE	
Dose:	0-10 MG/ML (TEST MATERIAL SOLVENT: DMSO)	
Results:	NEGATIVE	
Reference:	[DE VEER,I, MORISKE,HJ AND RUEDEN,H; PHOTOCHEMICAL DECOMPOSITION OF ORGANIC COMPOUNDS IN WATER AFTER UV-IRRADIATION: INVESTIGATION OF POSITIVE MUTAGENIC EFFECTS; TOXICOL. LETT. 72(1-3):113-119, 1994]	

As taken from CCRIS, 1995.

Type of Test	**Route of Exposure**	**Species Observed**	**Dose Data**	**Reference**
Dominant lethal test	Oral	Rodent - rat	54600 mg/kg/10W (continuous)	ENMUDM Environmental Mutagenesis. (New York, NY) V.1-9, 1979-87. For publisher information, see EMMUEG. Volume(issue)/page/year: 8,357,1986

As taken from RTECS, 2013.

Gum Arabic

Species/Cell Type:	Nonhuman
Assay Type:	Sister-chromatid exchange (SCE) in vivo
Assay Code:	SC3T
Results:	No conclusion

Reference:	EMICBACK/48665; MUTAT RES 113:33-43,1983

As taken from GENE-TOX, 1998

“ Based on the data available, the Panel considered that there is no concern with respect to the genotoxicity of acacia gum.”

As taken from EFSA, 2017

“BACKGROUND: Lifestyle-related diseases such as diabetes are steadily increasing worldwide. In Sudan, there are a variety of plant species used traditionally for the treatment of diabetes, obesity and other symptoms which need to be validated through scientific studies for their claimed traditional uses. Therefore, in the current study, the free radical scavenging activity, α-glucosidase inhibitory and pancreatic lipase inhibitory activities of 70% ethanol and water extracts of eighteen Sudanese medicinal plants were investigated using various in vitro assays. Moreover, the cytotoxicity and genotoxicity were assessed for the bioactive plant extracts. METHODS: Eighteen plants were selected on the basis of their traditional uses and extracted with 70% ethanol and water to obtain thirty-six extracts. The obtained extracts were screened using different in vitro bioassays namely, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, α-glucosidase inhibitory and pancreatic lipase inhibitory assays. Furthermore, the active plant extracts were investigated for their cytotoxicity and genotoxicity on HeLa cell line using HCS DNA Damage Assay. RESULTS: Both 70% ethanol and water extracts of Acacia nilotica, Ziziphus spina-christi, Abrus precatorius, and Geigeria alata along with the 70% ethanol extract of Martynia annua showed potent free radical scavenging activity. Regarding the α-glucosidase inhibition assay, both extracts of Acacia nilotica, Ziziphus spina-christi, Geigeria alata, and Cyperus rotundus showed potent activity. In general, 70% ethanol extracts were more potent compared to water extracts with exception of Cordia sinensis and Cymbopogon proximus, for which water extracts also showed potent enzyme inhibitory activity. Similarly, water extracts of Acacia nilotica and Ziziphus spina-christi showed potent inhibitory activity against pancreatic lipase enzyme. Some of the extracts also showed significant genotoxicity and cytotoxicity at the concentration range used for bioactivities. CONCLUSION: The extracts of Acacia nilotica, Ziziphus spina-christi, Geigeria alata, Martynia annua and Abrus precatorius exhibited an appreciable range of activity on antioxidant and enzyme inhibitory assays.” As taken from Elbashir SMI et al. 2018. BMC Complement. Altern. Med. 18(1), 282. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30340582

“Acetamiprid (ACE), a neonicotinoid insecticide, is widely used in agriculture either alone or in combination with other insecticides. A combined approach employing micronucleus test (MNT) and chromosomal aberrations (CA) assay was utilized to assess the genotoxic effects of ACE in bone marrow of Swiss albino male mice. Acetamiprid was administered i.p. daily at 4.6 and 2.3 mg/kg/day along with 3% gum acacia as negative control for 60 and 90 days and cyclophosphamide (50 mg/kg b.wt.) as positive control. ACE treatment resulted in a dose-dependent increase in the frequencies of micronuclei per cell and chromosomal aberrations in bone marrow cells. The increased micronuclei formation in total erythrocyte cells (immature PCEs and mature NCEs) was observed only at higher dose level (4.6 mg/kg b.wt.) administered for 90 days. The test also indicated the cytotoxic effect of higher dose level of pesticide by PCE/NCE ratio. The number of chromosomal aberrations were increased in the pesticide treated group compared to the negative control group, although significant increase was observed only in the group exposed to higher dose level of pesticide for both 60 and 90 days. Thus, daily exposure of ACE at a dose level of 4.6 mg/kg body weight for 60 and 90 days caused genotoxic and cytotoxic effects on the somatic cells of Swiss albino male mice.” As taken from Bagri P and Jain SK 2019. Drug Chem. Toxicol. 42(4), 357–363. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/29405074/

“Arabic gum (Acacia senegal, AG) is proven effective antioxidant and cytoprotective agent. The present study was designed to test this notion by investigating the possible role of AG against the radiographic contrast medium (Ioxitalamate, Telebrix-35®, TBX)-induced oxidative stress and

genotoxicity. Albino rats were divided into four groups and supplied with either; distilled water, daily 10% (w/v) AG, an intravenous dose of TBX (1600 mg l/kg b.wt) and co-administration of TBX and AG. Rats were sacrificed and blood samples were collected to assess the genotoxicity employing the peripheral blood leucocytes fluorescent double staining; namely the acridine orange/ethidium bromide (AO/EB) staining and alkaline comet assay. Further, chromosomal analyses were done in bone marrow cells. Serum urea and creatinine levels, in addition to malondialdehyde (MDA), nitric oxide (NO), catalase (CAT) and glutathione (GSH) levels in kidney tissues were measured. Liquid chromatography-mass spectrophotometry (LC-MS-MS) was performed to identify the chemical composition of AG extract. Kidney functions, single/double-stranded DNA damage, chromosomal aberrations, mitotic index, MDA and NO levels were significantly ($p < 0.001$) increased in TBX-treated group compared to the control and AG-treated one. Meanwhile, CAT and GSH activities were significantly diminished and the AG supplementation significantly ($p < 0.001$) ameliorated these effects compared with the control and AG-treated groups. Five compounds have been identified using GNPS networking including 7,3',4'-Trihydroxyisoflavone, Noscapine, Tetrahydropapaveroline, Costunolide, Hesperidin. In conclusion, results of the present study suggest that AG exerted a protective role against TBX-induced oxidative stress and genotoxicity which may be attributed to the active metabolites in the gum." As taken from El-Garawani I et al. 2021. Antioxidants (Basel) 10(2), 221. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33540787/

5.5. Cytotoxicity

Iron oxide magnetic nanoparticles (MNPs) were synthesized by the chemical co-precipitation method and coated with gum arabic (GA) by physical adsorption and covalent attachment. Cultures of mammalian cell lines (HEK293, CHO and TE671) were grown in the presence of uncoated and GA-coated MNPs. Cellular growth was followed by optical microscopy in order to assess the proportion of cells with particles, alterations in cellular density and the presence of debris. The in vitro assays demonstrated that cells from different origins are affected differently by the presence of the nanoparticles. Also, the methods followed for GA coating of MNPs endow distinct surface characteristics that probably underlie the observed differences when in contact with the cells. In general, the nanoparticles to which the GA was adsorbed had a smaller ability to attach to the cells' surface and to compromise the viability of the cultures. (Bicho A. et al., 2010).

"Acacia arabica and Moringa oleifera are credited with a number of medicinal properties. Traditionally gum of Acacia plant is used in the treatment of skin disorders to soothe skin rashes, soreness, inflammation and burns while Moringa seed extracts are known to have antibacterial activity. In the present study the potential of the polymeric component of aqueous extracts of gum acacia (GA) and the seeds of M. oleifera (MSP) in wound management was evaluated. The results revealed that both biopolymers were hemostatic and hasten blood coagulation. They....were non-cytotoxic in nature. Both showed antibacterial activity against organisms known to be involved in wound infections with MIC ranging from 500-600 microg mL(-1) for GA and 300-700 microg mL(-1) for MSP...." As taken from Bhatnagar M et al. 2013. Indian. J. Exp. Biol. 51(10), 804-10. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24266104

"Background: The herbal extracts have been effectively tried in the treatment and prevention of many oral diseases. Aim: The aim is to assess the antimicrobial efficacy of Acacia nilotica, Murraya koenigii L. Sprengel, Eucalyptus hybrid, Psidium guajava extracts and their combinations on Fusobacterium nucleatum (Fn) and Porphyromonas gingivalis (Pg). Materials and Methods: The extraction process was carried out by Soxhlet apparatus using ethanol as solvent. The combinations of the four plant extracts were prepared by combining an equal quantity of 10% solution of each of the four plant extracts. The antimicrobial efficacy testing of the plant extracts and their combinations on Fn and Pg was performed using agar well diffusion method. Columbia 5% of sheep blood agar plates were used for antimicrobial efficacy testing under anaerobic conditions. The qualitative assay was carried out to identify the various phytochemical constituents. Dimethyl

sulfoxide and 0.2% chlorhexidine acted as negative and positive controls, respectively. The mean diameter of inhibition zone between different categories was compared using one-way analysis of variance. Results: All the individual plant extracts and their double, triple, and quadruple combinations were effective in inhibiting the growth of these bacteria. However, 0.2% chlorhexidine produced the highest mean diameter of inhibition zone. Conclusion: The plant extracts in combinations offer enhanced antimicrobial efficacy due to their synergistic action besides slowing the development of bacterial resistance. Hence, these extracts in combinations could be used tried as effective alternates to chlorhexidine." As taken from Chandra Shekar BR et al. 2018. Indian J. Dent. Res. 29(5), 641-645. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30409946

"ETHNOPHARMACOLOGICAL RELEVANCE: In Mexico, plants are an important element of traditional medicine, and many are considered part of Mexican cultural heritage from prehispanic and colonial times. Nevertheless, relatively few systematic scientific studies have been conducted to fully characterize the chemical composition and pharmacological activities of Mexican medicinal plants. Acacia farnesiana is used in Mexican traditional medicine to treat dysentery and tuberculosis and therefore could have bioactive compounds that may explain its traditional use. AIMS OF THE STUDY: i) To isolate and characterize the compounds from the hexanic, chloroformic and methanolic extracts; ii) to identify the volatile compounds from methylated hexanic and chloroformic extracts using GC-FID and GC-MS methods; iii) to identify the compounds from methanolic and aqueous extracts using HPLC-Q-TOF-MS; iv) to test the activity of extracts and isolated compounds against Mycobacterium tuberculosis and dysentery bacteria. MATERIAL AND METHODS: A. farnesiana fruits were collected in Acatlán de Osorio, Puebla, Mexico. Hexanic, chloroformic, methanolic and aqueous extracts were prepared and analyzed by different chromatographic techniques including column chromatography, flash chromatography, GC-FID, GC-MS and HPLC-Q-TOF-MS. Structural elucidation was carried out by NMR spectroscopic analysis. The activity of extracts, phytochemicals and semi-synthetic derivatives against Mycobacterium tuberculosis H37Rv and G122 as well as dysentery bacteria (Campylobacter jejuni, Shigella flexneri, Salmonella enteritidis, Yersinia enterocolitica and enterohemorrhagic Escherichia coli) was determined by the broth microdilution method and reported as minimal inhibitory concentration (MIC μg/mL). RESULTS: From both hexane and chloroform extracts, tetracosanoic acid (2S)-2,3-dihydroxypropyl ester (1) and (3β,22E)-estigmasta-5,22-dien-3-yl β-D-glucopyranoside (2) were isolated and characterized. From the methanolic extract, methyl gallate (3), gallic acid (4), (3β,22E)-estigmasta-5,22-dien-3-yl β-D-glucopyranoside (2), (2S) naringenin 7-O-β-glucopyranoside (prunin, 5), pinitol (6) and sucrose (7) were isolated and characterized. Furthermore, hexanic and chloroformic extracts were analyzed by GC-FID and GC-MS and 18 methylated fatty acids were identified for each extract in addition to three sterols. The methanolic and aqueous extracts were analyzed separately by HPLC-Q-TOF-MS, and 15 compounds were identified in each extract. The compounds 1, 2, and 7, in addition to 13 fatty acids and eight phenolic compounds, were identified for the first time in A. farnesiana. The extracts showed antitubercular (MIC 100-200 μg/mL) and antidysentery activity (MIC 100-200 μg/mL). Methyl gallate and its acetylated derivative showed activity against the sensible strain M. tuberculosis H37Rv with MIC values of 50-25 μg/mL, respectively. The flavanone prunin showed activity against multidrug resistant M. tuberculosis G122 (MIC 50 μg/mL). Methyl gallate, gallic acid and prunin showed activity against C. jejuni (MIC 50 μg/mL). CONCLUSIONS: The activity of tested extracts and isolated compounds against M. tuberculosis and dysentery bacteria justifies the ethnomedical use of A. farnesiana fruits for the treatment of tuberculosis and dysentery." As taken from Hernández-García E et al. 2019. J. Ethnopharmacol. 230, 74-80. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30367988

"BACKGROUND: Despite the significant developments occurring in the treatment of cancer, it still remains the second deadly disease, responsible for 8.2 million deaths every year. Various natural substances have been studied for active molecules of tumor suppression in the past and the

tropical flora, by its diversity, continues to provide new antitumor drugs. Acacia seyal is a plant used in Cameroonian traditional system to treat cancer. It exhibited cytotoxic effects towards human breast adenocarcinoma cells. The present work was therefore designed to elucidate the underlying mechanisms by which A. seyal extract induced its cytotoxic effect. METHODS: The cell death mechanism (apoptosis or necrosis) and cell cycle analyses were assessed using flow cytometry. The levels of reactive oxygen species (ROS), mitochondrial membrane potential (ΔΨm), caspases activities as well as Bcl-2 and Bcl-xL protein contents were assessed in MDA-MB-231 cells. Afterwards, cell migration/invasion was also assessed. RESULTS: The A. seyal extract induced apoptosis in MDA-MB-231 cells, while it failed to do so in MCF-7 cells. It induced cell cycle arrest in G2/M phase. Further it induced a decrease in ΔΨm, an increase in ROS levels and caspases activities as well as a down regulation in Bcl-2 and Bcl-xL protein contents in MDA-MB-231 cells. Moreover, A. seyal extract exhibited anti-migration, anti-invasion activities in MDA-MB-231 cells. CONCLUSION: These results demonstrate that A. seyal extract induced its antitumor effects mainly by interference in metastasis related events, by triggering apoptosis through a ROS-mediated mitochondrial pathway." As taken from Zingue S et al. 2018. J. Ethnopharmacol. 223, 41-50. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29783017

"The Gum Arabic of Acacia senegal (GA) was reported to treat several diseases such as kidney failure, cardiovascular, and gastrointestinal. However, scarce investigations has been achieved on phytoconstitutes of GA. Therefore, the screening was carried out to study phytochemical properties. The phytochemical screening showed that GA contains high amount of saponins and alkaloids, moderate amount of cardiac glycosides, and trace amount of tannins. Consequently, the bioactivity of GA aqueous extract as antimicrobial and mosquito larvicides were investigated. GA extract exhibited antimicrobial activity against the test organisms with different zones of inhibition ranging 0-18 mm. The larvicidal activity was significantly improved with increasing extract dose and exposure period with mortality up to 86.7%. Results reveal that the crude extract of GA contains important biomolecules which proved with a substantial larvicidal and antimicrobial activities." As taken from Daffalla HM 2018. Walailak Journal of Science and Technology (WJST) 17. Available at: http://wjst.wu.ac.th/index.php/wjst/article/view/5540

"The present study aimed to assess the in vitro antibacterial and antibiotic modifying activities of methanol extracts prepared from the leaf (APL) and bark (APB) of Acacia polyacantha, fractions (APLa-d) and compounds isolated from APL against a panel of multidrug resistant (MDR) Gram-negative bacteria. Leaf extract was subjected to column chromatography for compounds isolation; antibacterial assays were performed on samples alone and with an efflux pump inhibitor (EPI), respectively, and several antibiotics on the tested bacteria. The phytochemical investigation of APL led to the isolation of stigmasterol (1), β-amyrin (2), 3-O-β-glucopyranosylstigmasterol (3), 3-O-methyl-D-chiro-inositol (4), epicatechin (5), quercetin-3-O-glucoside (6), 3-O-[β-xylopyranosyl-(1→4)-β-galactopyranosyl]-oleanolic acid (7), and 3-O-[β-galactopyranosyl-(1→4)-β-galactopyranosyl]-oleanolic acid (8). APL and APB had minimal inhibitory concentration (MIC) values ≤ 1024 μg/mL on 73.3% and 46.7% of the tested bacteria, respectively. APLb and APLd were effective against 88.9% of tested bacterial species with compound 8 showing the highest activity inhibiting 88.9% of tested bacteria. The EPI, phenylalanine-arginine-β-naphthylamide (PAßN), strongly improved the activity of APL, APLb, APLd, and compound 8 on all tested bacteria. Synergistic effects were obtained when APL and compounds 7 and 8 were combined with erythromycin (ERY), gentamycin (GEN), ciprofloxacin (CIP), and norfloxacin (NOR). The present study demonstrates the antibacterial potential of Acacia polyacantha and its constituents to combat bacterial infections alone or in combination with EPI." As taken from Mambe FT et al. 2019. Evidence-based complementary and alternative medicine. Article ID 7507549. Available at https://www.hindawi.com/journals/ecam/2019/7507549/cta/

"Gum Arabic as Prebiotic is food ingredients that stimulate the growth of useful bacteria which lives in the large intestine of the human being or animal since birth and beneficial to the digestive

system, body's immunity, disposal of poisons, fats and excreta. Besides it negates the effects of harmful bacteria thus protecting from the diseases, cancer, diabetes and obesity. Present study aimed to investigate the in vitro and in vivo the effect of Arabic gum as exudates and different parts of the tree extracts; Aqueous, ethanol and Hexsane extracts of exudates, leaf, fruits and Bark of Arabic Gum tree as potential antibacterial against different several strains of pathogenic bacteria it was concluded that There was a strong inhibitory effect of aqueous, ethanol and hexane extracts of Arabic Gum exudates, leaf, fruit and bark on most of the gram positive and gram negative bacteria. The largest inhibition zones of bacterial strains were for Staphylococcus aureus, followed by Streptococcus pyogenes and Salmonella typhimurium while the smallest inhibition zones were for Yersinia pestis, Pseudomonas aeruginosa and Staphylococcus epidermidis. The three tested strains greatly inhibited due to the presence of Arabic Gum in diet of mice but with different degrees, make us noticed that the most bacterial strain affected was Streptococcus pyogenes followed by Staphylococcus aureus followed by Salmonella typhimurium." As taken from Osman HAE-FM and Zaki MS, 2019. Researcher 11(2), 17-23. Available at http://www.sciencepub.net/researcher/rsj110219/05_34449rsj110219_17_23.pdf

"Infectious diseases are important cause of morbidity and mortality due to continuous emergence of microbial resistance to conventional drugs. Acacia nilotica, Ziziphus jujube Linn and Lawsonia inermis are widely used for traditional medicine in Northern Nigeria. However, little is known about the biochemical and microbiological potentials of these indigenous plants. In this study, the plants leaves were screened for phytochemical and in vitro antimicrobial potentials using standard methods. Quantitative phytochemical analysis of crude methanolic leave extracts revealed high content of glycoside, tannins and phenols. High levels of saponins and flavonoids were also detected. The extracts exhibited antibacterial effects on Escherichia coli, Pseudomonas flourecense, Streptococcus and Staphylococcus aureus. At 50 mg/ml extract concentration, the zone of inhibition observed was greater than 6mm. This indicates high inhibitory potency of the plants leaves. In comparison to streptomycin sulphate, A. nilotica and L. inermis had statistically similar ($P>0.05$) effect on E. coli at 50 mg/ml. In general, the inhibitory effect of A. nilotica and L. inermis were higher than that of Z. jujube Linn in all concentrations, except on E. coli at 150 mg/ml. Both the extracts and control drug had minimum inhibitory concentration of 10 mg/ml for all the microbes tested except Streptococcus (20-25 mg/ml). Furthermore, the average Minimum Bactericidal Concentration was 15 mg/ml except for Streptococcus with 20-25 mg/ml. Methanol extracts of Acacia nilotica, Ziziphus jujube Linn, and Lawsonia inermis exhibit antibacterial effect, hence could be used as sources of potent agents against bacterial infection." As taken from Abubakar AL et al. 2018. Nigerian Journal of Basic and Applied Science 26(2), 01-08. Available at https://www.ajol.info/index.php/njbas/article/view/184782

"Gum arabic (GA) is a traditional herbal medicine from Acacia Senegal (L.) Willdenow trees, which consist of a complex mixture of polysaccharides and glycoproteins. It is used in daily applications for several diseases and is considered to protect against bacterial infections. The detailed mechanisms behind these observations are still unclear. In this study, we investigated the direct antibacterial activity of GA water and ethanol extracts against Staphylococcus (S.) aureus or Escherichia (E.) coli and the immunomodulating properties of those extracts on granulocytes as a first line of defense against bacteria. Firstly, the direct antimicrobial effect of GA was tested on three different S. aureus strains and two E. coli strains. The growth of bacteria was analyzed in the presence of different GA concentrations over time. GA water as well as ethanol extracts showed a significant growth inhibition in a concentration-dependent manner in the case of S. aureus Newman, S. aureus Rd5, and E. coli 25922, but not in the case of S. aureus USA300 and E. coli K1. Transmission electron microscopic analysis confirmed an antibacterial effect of GA on the bacteria. Secondly, the immunomodulatory effect of GA on the antimicrobial activity of bovine or human blood-derived granulocytes was evaluated. Interestingly, water and ethanol extracts enhanced antimicrobial activity of granulocytes by the induction of intracellular ROS production. In line with these data, GA increased the phagocytosis rate of E. coli. No effect was seen on

neutrophil extracellular trap (NET) formation that mediates killing of extracellular bacteria such as S. aureus. In conclusion, we show that GA exhibits a direct antibacterial effect against some S. aureus and E. coli strains. Furthermore, GA boosts the antimicrobial activities of granulocytes and increases intracellular ROS production, which may lead to more phagocytosis and intracellular killing. These data might explain the described putative antimicrobial activity of GA used in traditional medicine." As taken from Baien SH et al. 2020. Front. Immunol. 10, 3119. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/32082302/

"Gum Arabic is a natural gummy exudate gained from the trees of Acacia species (Acacia senegal and Acacia seyal), Family: Fabaceae. Gum Arabic considers as a dietary fiber with a high percentage of carbohydrates and low protein content. Sugars arabinose and ribose were originally discovered and isolated from gum Arabic and it is representing the original source of these sugars. A gum emanation from trees occurs under stress conditions such as heat, poor soil fertility, drought, and injury. Mainly gum is produced in belt region of Africa, mainly Sudan, Chad, and Nigeria. In the food industry, it is used in confectionery; in the pharmaceutical industry, it is used as emulsifier, film coating and others. Traditionally the gum used for chronic renal failure, digestive discomfort, and others. Although gum Arabic considered as an inert substance, recent information demonstrated multiple pharmacological and medical effects, such as weight reduction, antihypertensive, antihyperlipidemic, anticoagulant, antibacterial, antidiabetic, anti-inflammatory, nephroprotective and other effects." As taken from Jaafar NS. 2019. Iraqi J. Pharm. Sci. 28(2), 9-16. Available at http://bijps.uobaghdad.edu.iq/index.php/bijps/article/view/896

5.6. Carcinogenicity

... Under the conditions of this bioassay, gum arabic was not carcinogenic for F344 rats or B6C3F1 mice of either sex. ... Levels of Evidence of Carcinogenicity: Male Rats: Negative; Female Rats: Negative; Male Mice: Negative; Female Mice: Negative [Carcinogenesis Bioassay of Gum Arabic in F344 Rats and B6C3F1 Mice. Technical Report Series No. 227 (1982) NIH Publication No. 82-1783 U.S. Department of Health and Human Services, National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709] **PEER REVIEWED** A carcinogenesis bioassay of gum arabic (81 -86% pure) ... was conducted by feeding diets containing 25,000 or 50,000 ppm of the test substance to 50 F344 rats and 50 B6C3F1 mice of each sex for 103 wk. Groups of untreated rats and mice of each sex served as controls. ... Under the conditions of this bioassay, gum arabic was not carcinogenic for F344 rats or B6C3F1 mice of either sex. ... Levels of Evidence of Carcinogenicity: Male Rats: Negative; Female Rats: Negative; Male Mice: Negative; Female Mice: Negative. [Carcinogenesis Bioassay of Gum Arabic in F344 Rats and B6C3F1 Mice. Technical Report Series No. 227 (1982) NIH Publication No. 82-1783 U.S. Department of Health and Human Services, National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709] **PEER REVIEWED**

As taken from HSDB, 2002.

Mouse: Groups of 50 male and 50 female B6C3F1 mice were given arabic gum in the diet at concentrations of 0, 25 000 or 50 000 ppm (0, 2.5 or 5.0%) for 103 weeks. The animals were maintained on a control diet for an additional 2 weeks prior to sacrifice. No effect of the test compound on body weight gain was noted in either sex, although mean daily food consumption was reduced in both sexes of the groups receiving arabic gum. No effect of the test compound was noted with respect to survival, clinical signs, or incidence of gross or microscopic non-neoplastic lesions. Hepatocellular adenoma of the liver was found in 2/49 controls, 0/50 low-dose and 6/49 high-dosefemales. Hepatocellular carcinomas were found in 1/49 controls, 2/50 low-dose and 6/50 high-dose females. The number of female mice with hepatocellular adenoma, carcinoma or unspecified neoplasm of the liver were 4/49, 2/50 and 10/49 in controls, low-dose and high-dose animals, respectively. Some high-dose animals had both a hepatocellular adenoma and a hepatocellular carcinoma. The historical records at the performing laboratory indicate the incidence

of control female B6C3F1 mice with adenomas or carcinomas of the liver has been 56/975 (5.7%) with a range of 1/50 to 11/54 (2-20.3%). Male mice given arabic gum did not have an increased incidence of liver tumours. The incidence of mice with haemangiomas or haemangiosarcomas of the circulatory system was not significant in either sex. The conclusion of the study was that there was no site at which an increase in tumour incidence could be clearly associated with the administration of the chemical (National Toxicology Program, 1980).

Rat: Groups of 50 male and 50 female Fischer 344 rats were given gum arabic in the diet at concentrations of 0, 25 000 or 50 000 ppm (0, 2.5 or 5.0%) for 103 weeks. The animals were maintained on the control diets for an additional 2 weeks prior to sacrifice. In the males, body weights of test and control animals were comparable throughout the study, while in the females, weight gain in the test animals was slightly less than that of controls. The effect was not dose related. As compared to controls, feed intake was reduced in test males and test females. No effects of the test compound were reported with respect to clinical signs, survival, or incidence of gross or microscopic lesions (National Toxicology Program, 1980).

As taken from JECFA, 1982.

Carcinogenicity Studies:

Species:	MOUSE
Strain/Sex:	B6C3F1/FEMALE
Route:	ORAL
Dose:	0; 25000; 50000 PPM IN DIET
Results:	NEGATIVE
Reference:	[NCI/NTP CARCINOGENESIS TECHNICAL REPORT SERIES; NATIONAL CANCER INSTITUTE/NATIONAL TOXICOLOGY PROGRAM; U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, TR-227 Y82]
Species:	MOUSE
Strain/Sex:	B6C3F1/MALE
Route:	ORAL
Dose:	0; 25000; 50000 PPM IN DIET
Results:	NEGATIVE
Reference:	[NCI/NTP CARCINOGENESIS TECHNICAL REPORT SERIES; NATIONAL CANCER INSTITUTE/NATIONAL TOXICOLOGY PROGRAM; U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, TR-227 Y82]
Species:	RAT
Strain/Sex:	F344/FEMALE
Route:	ORAL
Dose:	0; 25000; 50000 PPM IN DIET
Results:	NEGATIVE
Reference:	[NCI/NTP CARCINOGENESIS TECHNICAL REPORT SERIES; NATIONAL CANCER

	INSTITUTE/NATIONAL TOXICOLOGY PROGRAM; U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, TR-227 Y82]
Species:	RAT
Strain/Sex:	F344/MALE
Route:	ORAL
Dose:	0; 25000; 50000 PPM IN DIET
Results:	NEGATIVE
Reference:	[NCI/NTP CARCINOGENESIS TECHNICAL REPORT SERIES; NATIONAL CANCER INSTITUTE/NATIONAL TOXICOLOGY PROGRAM; U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, TR-227 Y82]

As taken from CCRIS, 1995.

EFSA documentation stated ‘that studies on carcinogenicity of gum arabic (acacia gum) in mouse (B6C3F, both sexes) and rat (F344, both sexes) are available. As regards the mouse, in a 103-weeks feeding study with acacia gum at levels up to 5% in the diet (equivalent to about 7500 mg/kg bw/day), there was no site at which an increase in tumour incidence could be clearly associated with the administration of the chemical (NTP, 1982). Similarly in a 103-week feeding study in the rat with acacia gum at levels up to 5% in the diet (equivalent to about 2500 mg/kg bw/day), no effects of the test compound were reported with respect to clinical signs, survival, or incidence of gross or microscopic lesions (NTP, 1982), (EFSA, 2010a).

“Gum arabic (GA), a water-soluble dietary fiber rich in Ca(2+), Mg(2+) and K(+), is used in Middle Eastern countries for the treatment of patients with chronic kidney disease....GA treatment counteracts the development of tumors following chemical cancerogenesis....” As taken from Nasir O. 2013. Kidney Blood Press. Res. 37(4-5), 269-79. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24022265

“No chronic toxicity studies according to OECD guidelines (452) or equivalent have been identified. Acacia gum was tested for carcinogenicity in rats and mice receiving diets containing 2.5% and 5% acacia gum in the feed for 103 weeks equivalent to 1,250 and 2,500 mg acacia gum/kg bw per day in rats, and 3,750 and 7,500 mg acacia gum/kg bw per day in mice (NTP, 1982; Melnick et al., 1983). From this study, the Panel considered that acacia gum is not of concern with respect to carcinogenicity.”

As taken from EFSA, 2017

5.7. Irritation/immunotoxicity

When applied locally to irritated or abraded tissues, demulcents tend to coat surface &, by mechanical means, protect underlying cells from stimuli that result from contact with air or irritants in environment. /demulcents/ [Goodman, L.S., and A. Gilman. (eds.) The Pharmacological Basis of Therapeutics. 5th ed. New York: Macmillan Publishing Co., Inc., 1975., p. 946] **PEER REVIEWED**

Printing workers exposed to.../gum arabic/ mist have been found to suffer from allergic reactions often known as "printer's asthma", frequency of allergic symptoms depending mainly on atmospheric gum arabic concn. [International Labour Office. Encyclopedia of Occupational Health and Safety. Volumes I and II. New York: McGraw-Hill Book Co., 1971., p. 629] **PEER REVIEWED**

...study of printers exposed to gum... on the avg, 5 years or more passed before asthma occurred...printers with personal family history of atopic allergy developed symptoms much sooner. ...one firm in which 30%...complained of wheezing and 19%...asthma. [Hamilton, A., and H. L. Hardy. Industrial Toxicology. 3rd ed. Acton, Mass.: Publishing Sciences Group, Inc., 1974., p. 461] **PEER REVIEWED**

Further studies have shown...sensitization occurred in about 50% of workers. course of allergy is in 2 periods...first antigen-antibody reaction sometimes without symptoms (silent sensitization). this is followed in many cases by clinical disorders due to another mechanism... [International Labour Office. Encyclopedia of Occupational Health and Safety. Volumes I and II. New York: McGraw-Hill Book Co., 1971., p. 629] **PEER REVIEWED**

As taken from HSDB, 2002.

Allergenic reactions have been reported in man following ingestion of the substance.

As taken from JECFA, 1982.

“Sensitivity to arabic gum was found to be a true antigen-antibody response in the guinea-pig (Rice, 1955; Silvette et al., 1955).”“Sensitivity to arabic gum as a tablet additive has been reported in some kidney transplant patients. Hypersensitivity manifested as itching, and rash with fever and arthralgia was also reported in individual patients (Rubinger et al. 1978). Sensitivity of some individuals to gum arabic in food has also been reported (Gelfand, 1949).”

Gum Arabic as a Cause of Occupational Allergy (Abstract)

Background. Gum arabic is a potential sensitizer in food industry. Methods. We examined 11 candy factory workers referred to examinations due to respiratory and skin symptoms paying attention to exposure and sensitization to gum arabic. Skin tests, pulmonary function tests, and respiratory provocation tests were carried out as indicated by the symptoms and findings. Results. Occupational asthma, caused by gum arabic was diagnosed in 4/11 candy factory workers and two of them had also occupational contact urticaria and one had occupational rhinitis. One of them had oral symptoms associated with ingestion of products containing gum arabic. Conclusions. Airborne exposure to gum arabic may cause sensitization leading to allergic rhinitis, asthma, and urticarial. As taken from Viinanen et al., (2011).

“Gum arabic (GA), a water-soluble dietary fiber rich in Ca(2+), Mg(2+) and K(+), is used in Middle Eastern countries for the treatment of patients with chronic kidney disease....In mouse dendritic cells, antigen-presenting cells linking innate and adaptive immunity, GA treatment modifies maturation and cytokine release. GA treatment further favourably influences the course of murine malaria....the effects of GA on angiogenin expression and dendritic cells may be useful in the treatment of inflammatory disease and malaria.” As taken from Nasir O. 2013. Kidney Blood Press. Res. 37(4-5), 269-79. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24022265

Type of Test	Route of Exposure	Species Observed	Dose Data	Reaction Severity	Reference
Standard Draize test	Administration into the eye	Rodent - rabbit	36 mg/5H	Severe	AROPAW Archives of Ophthalmology (Chicago). (AMA, 535 N. Dearborn St., Chicago, IL 60610) New series: V.1-44(3), 1929-50; V.64- 1960- Volume(issue)/page/year: 78,384,1967

As taken from RTECS, 2013.

Gum Arabic is a “skin, eye, mucous membrane, and upper respiratory tract irritant; a weak allergen that may cause local contact dermatitis and respiratory symptoms on inhalation of dust.”

“Occupational asthma reported in printer.”

“Occupational diseases associated with exposure to this agent: Asthma, occupational”

As taken from Haz-Map, 2021.

“No case reports on allergic reaction after oral exposure to acacia gum could be identified by the Panel.”

As taken from EFSA, 2017

“Thaumatin is a sweetener and flavor modifier commonly used in the food industry. Likewise, gum arabic is widely used as a food stabilizer and thickening agent. We report here that a powder mixture composed of 10% thaumatin and 90% gum arabic led to allergic symptoms in the upper airways in occupationally exposed individuals: four of eight workers of a chewing gum factory exposed to this powder mixture had pronounced rhinitis. A positive skin prick test result for pure thaumatin was obtained in all four individuals with rhinitis of whom two also had a positive skin prick test result for pure gum arabic and gum arabic-specific IgE. Subsitution of a powdered thaumatin with a liquid form reduced symptoms among the rhinitic workers. Although gum arabic is a well-known potential allergen, we were unable to find prior documentation of allergic symptoms to thaumatin when it is used in the food industry.” As taken from Tschannen MP et al. 2017. Am. J. Ind. Med. 60(7), 664-669. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28543634

“Gum arabic and cashew nut tree gum exudate polysaccharide (CNTG) are plant polysaccharides composed of galactose and arabinose known as arabinogalactans (AGs). Although these fractions are used in food and pharmaceutical industry, cases of allergic reactions were described in clinical reports. As AGs were reported as modulators of the classical (CP) and alternative pathways (AP) of complement system (CS), in the present work, we investigate whether gum arabic and CNTG have an effect on both CS pathways. The complement fixation tests were performed with (CP-30 and AP-30) and without pre-incubation (CP-0 and AP-0). For CP-30, CNTG and gum arabic (833 μg/ml) showed a reduction of 28.0% (P = 0.000174) and 48.5% (P = 0.000143), respectively, on CP-induced haemolysis. However, no effect was observed for CP-0 in the CP-induced haemolysis. For AP-30, both CNTG and gum arabic (833 μg/ml) showed 87% reduction on the CP-induced haemolysis, with IC50 values of 100 and 7 μg/ml, respectively. For AP-0, a reduction of 11.3% for gum arabic and no effect for the CNTG on the CP-induced haemolysis were observed. These results suggested that gum arabic and CNTG could be acting as activators of the CS. Thus, this effect on the CS, especially on the AP, which accounts for up to 80-90% of total CS activation, indicates that both fractions may be harmful because of their potential pro-inflammatory action. Considering that CS activation induces inflammatory response, further studies confirming this immunomodulatory effect of these fractions are required to insure their safe use.” As taken from Bovo F et al. 2016. Scand. J. Immunol. 83(5), 314-20. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26972106

“This study investigated the effects of proanthocyanidins derived from Acacia (Acacia mearnsii) bark extract in healthy Japanese adult subjects experiencing uncomfortable skin symptoms. All subjects were randomly allocated into two groups (n = 33 each) using a computerized random-number generator. The subjects received either Acacia bark extract tablets or placebo for 8 weeks. Evaluations included water content in the stratum corneum, transepidermal water loss (TEWL), Skindex-16, dermatology life quality index (DLQI), visual analog scale for desire to scratch, and blood tests. At 4 weeks, the symptom/feeling score of DLQI, subjective symptoms related to uncomfortable skin, and the desire to scratch were significantly reduced in the intervention group than in the placebo group. At 8 weeks, the intervention group exhibited significantly lower TEWL on facial skin than that in the placebo group. In conclusion, the intake of Acacia bark extract tablets reduced TEWL and improved dry and uncomfortable skin.” As taken from Hoshino T et al. 2019. Biosci. Biotechnol. Biochem. 83(3), 538-550. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30526388

„Gum Arabic (GA) is a plant exudate with antioxidant and anti-inflammatory effects. GA has shown promise in protection from and treatment of kidney failure, however, its role in the protection of the heart from ischemia and reperfusion (I/R) has not been investigated. This study investigated the antioxidant and anti-inflammatory effects of Gum Arabic (GA) in the protection of the heart against ischemia/reperfusion (I/R) injury. Langendorff-perfused Wistar rat hearts were divided into seven groups. One group which was subjected to I/R with no other treatment served as the control group. The second group was subjected to buffer perfusion with no ischemia (sham group). The third group was perfused with GA in the absence of ischemia (sham + GA). The rest of the hearts were isolated from rats that had been treated with GA for 4 or 2 weeks in the drinking water, or GA that had been infused intravenously 2 h before sacrifice or added to perfusion buffer at reperfusion. Hemodynamics data were digitally computed; infarct size was measured using 2,3,5-triphenyltetrazolium chloride (TTC) staining and cardiomyocyte injury was assessed by quantifying creatine kinase (CK) and lactate dehydrogenase (LDH) enzymes. The total oxidants (TOS) and antioxidants (TAS), superoxide dismutase (SOD) and pro- and anti-inflammatory cytokines levels were estimated by ELISA. GA treatment for 2 weeks, 4 weeks or 2 hours before sacrifice resulted in a significant ($P < 0.05$) improvement in cardiac hemodynamics and reduction in infarct size and cardiac enzyme levels compared to respective controls. However, GA administration at the time of reperfusion did not protect the hearts against I/R injury. Furthermore, GA treatment decreased the pro-inflammatory and anti-inflammatory cytokines levels. The levels of TOS in the effluent were significantly decreased ($P < 0.05$) and SOD levels were significantly ($P < 0.05$) increased by GA administration. GA protected the heart against I/R injury when administered for 2 or 4 weeks or when infused 2 hours before sacrifice. GA treatment decreased the total oxidants levels, the pro-inflammatory cytokines TNF-α, IL-1β and IL-6 protein levels and increases SOD and anti-inflammatory cytokine IL-10 protein levels."
As taken from Gouda E. (2022). Available at https://pubmed.ncbi.nlm.nih.gov/36241904/

5.8. All other relevant types of toxicity

"BACKGROUND: Diabetes mellitus is a chronic metabolic disease with life-threatening complications. Despite the enormous progress in conventional medicine and pharmaceutical industry, herbal-based medicines are still a common practice for the treatment of diabetes. This study evaluated ethanolic and aqueous extracts of selected Sudanese plants that are traditionally used to treat diabetes. METHODS: Extraction was carried out according to method described by Sukhdev et. al. and the extracts were tested for their glycogen phosphorylase inhibition, Brine shrimp lethality and antioxidant activity using (DPPH) radical scavenging activity and iron chelating activity. Extracts prepared from the leaves of Ambrosia maritima, fruits of Foeniculum vulgare and Ammi visnaga, exudates of Acacia Senegal, and seeds of Sesamum indicum and Nigella sativa. RESULTS: Nigella sativa ethanolic extract showed no toxicity on Brine shrimp Lethality Test, while its aqueous extract was toxic. All other extracts were highly toxic and ethanolic extracts of Foeniculum vulgare exhibited the highest toxicity. All plant extracts with exception of Acacia senegal revealed significant antioxidant activity in DPPH free radical scavenging assay. CONCLUSIONS: These results highly agree with the ethnobotanical uses of these plants as antidiabetic. This study endorses further studies on plants investigated, to determine their potential for type 2 diabetes management. Moreover isolation and identification of active compounds are highly recommended." As taken from Hilmi Y et al. 2014. BMC Complement. Altern. Med. 14, 149. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/24885334

"In humans, the repeated oral daily intake of a large amount of acacia gum up to 30 g (approx. 430 mg acacia gum/kg bw per day) for up to 18 days was well tolerated and had only a minimum effect on stool weight and decrease in serum cholesterol. Some individuals experienced flatulence and this "was considered by the Panel as undesirable but not adverse".

"A dose of 53,000 mg acacia gum/person per day (approximately equivalent 760 mg acacia gum/kg bw per day) induced mild flatulence."

As taken from EFSA, 2017, 2019.

“BACKGROUND: Sickle cell anemia patients suffer from oxidative stress due to chronic inflammation and self-oxidation of sickle hemoglobin (Hb S). Chronic oxidative stress contributes to endothelial dysfunction, inflammation and multiple organ damage in sickle cell disease (SCD). Thus, antioxidant medication may favorably influence the disease. Gum Arabic (GA), edible, dried, gummy exudates from Acacia Senegal tree, has been claimed to act as an anti-oxidant and cytoprotective agent, protecting against experimental hepatic, renal and cardiac toxicities in rats. We hypothesized that regular intake of GA increases anti-oxidant capacity and reduce oxidative stress. METHODS: Forty-seven patients (5-42 years) carrying hemoglobin SS were recruited. Patients received 30 g/day GA for 12 weeks. Total anti-oxidant capacity (TAC), malondialdehyde (MDA) and hydrogen peroxide (H2O2) levels were measured by spectrophotometric methods before and after GA intake. Complete blood count was measured by sysmex. RESULTS: Gum Arabic significantly increased TAC level $P < 0.001$and decreased the oxidative markers MDA ($P < 0.05$) and H2O2 ($P < 0.005$). CONCLUSIONS: GA has potent anti-oxidative properties in sickle cell anemia. The anti-oxidant effect of GA may thus favorably influence the clinical condition of this and further diseases characterized by oxidative stress.” As taken from Kaddam L et al. 2017. BMC Hematol. 17, 4. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28331623

“Purpose: To evaluate red propolis, gum arabic and L-lysine activity on colorectal preneoplastic lesions induced by azoxymethane (AOM). Methods: The study featured 4 control groups (I-IV) and 4 experimental groups (V-VIII), totaling 48 rats. Once a week for 2 weeks, animals on control groups received saline, while animals in experimental groups received azoxymethane (15 mg/kg i.p.). The follow up along 16 weeks included daily oral gavage to administer water (I and V), L-lysine (150 mg/kg)(II and VI), própolis (100mg/5ml/kg)(III and VII), or gum arabic (5ml/kg)(IV and VIII). Was performed surgery on the animals in the end of this time in order to collect blood for biological assays (TBARS, GSH), followed by their sacrifice to tissue extract. Results: Oxidative stress (TBARS) and the number of aberrant crypt foci (ACF) in distal colon were lower using própolis ($p<0.01$ for both parameters). Gum arabic reduced preneoplastic lesions (ACF ≤ 4 crypts) on distal colon and on the entire colon ($p<0.05$). Conclusions: Red propolis reduced AOM-induced oxidative stress (TBARS) and total number of ACF in the distal colon. L-lysine neither protected against nor enhanced AOM-induced ACF. Gum arabic reduced the number of ACF.” Braga VNL et al. 2019. Acta Cir. Bras. 34(2), e201900207.

“In the study of Bliss et al. (2001), patients with stool incontinence were treated with psyllium, gum arabic, or a placebo for 31 days after a run-in period of 8 days. From 42 patients (age 34–76 years; 62 ± 3 (mean ± SEM) years) recruited for the study 39 completed the study according to the protocol. The dose of gum arabic was 25 g/day given mixed with half-strength fruit juice in two servings (morning and evening). Whereas the proportion of ‘incontinent’ stools was significantly less in the gum arabic group compared to the control, no influence on the frequency of flatus (in the initial period the dose was given in increasing doses until after 6 days the full dose was reached) and on SCFAs [short chain fatty acid] concentration in stool was observed.

In the double-blinded, controlled study of Calame et al. (2008) 54 healthy volunteers (age 30.6 ± 13 years) were randomly assigned to six groups being treated with water as control, or 5, 10, 20 and 40 g of EmulGold (which is a tradename of gum arabic) per day or inulin as positive control (10 g Fibruline per day which is the tradename) over 4 weeks. The primary endpoint was the change in microbiota whereby the genera of Bifidobacteria and lactobacilli were taken as potentially beneficial bacteria and those of Bacteroides, Clostridium difficile and enterococci as potentially non-beneficial. The secondary endpoint was side effects, in particular diarrhoea. There were some statistically significant changes in the numbers of Bifidobacteria and lactobacilli (increase at 10 g/day) and also in Bacteroides (increase at 10 g/day). The Panel considered the changes as not clinically meaningful. Gastrointestinal side effects, in particular diarrhoea, did not occur more frequently in all treated groups compared to control.

In summary, the two studies in adults did not show adverse effects of gum arabic up to a dose of 40 g/day (0.64 g/kg bw per day, calculated with the actual weight) in healthy volunteers treated over 4 weeks and up to 25 g/day (0.30 g/kg bw per day, calculated with the actual weight) in patients with stool incontinence. However, one study focuses on effects on the microbiome which cannot be evaluated at the present state of knowledge and the other was performed in patients with stool incontinence."

As taken from EFSA, 2019.

"Acacia gum (AG) is a non-viscous soluble fiber that is easily incorporated into beverages and foods. To determine its physiological effects in healthy human subjects, we fed 0, 20, and 40 g of acacia gum in orange juice along with a bagel and cream cheese after a 12 h fast and compared satiety, glycemic response, gastrointestinal tolerance, and food intake among treatments. Subjects (n = 48) reported less hunger and greater fullness at 15 min (p = 0.019 and 0.003, respectively) and 240 min (p = 0.036 and 0.05, respectively) after breakfast with the 40 g fiber treatment. They also reported being more satisfied at 15 min (p = 0.011) and less hungry with the 40 g fiber treatment at 30 min (p = 0.012). Subjects reported more bloating, flatulence, and GI rumbling on the 40 g fiber treatment compared to control, although values for GI tolerance were all low with AG treatment. No significant differences were found in area under the curve (AUC) or change from baseline for blood glucose response, although actual blood glucose with 20 g fiber at 30 min was significantly less than control. Individuals varied greatly in their postprandial glucose response to all treatments. AG improves satiety response and may lower peak glucose response at certain timepoints, and it is well tolerated in healthy human subjects. AG can be added to beverages and foods in doses that can help meet fiber recommendations." As taken from Larson R et al. 2021. Nutrients 13(2), 618. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33672963/

6. Functional effects on

6.1. Broncho/pulmonary system

Demulcents /eg acacia/ act by coating irritated pharyngeal mucosa & they may have brief antitussive effect on cough secondary to such irritation. /demulcents/ [american medical association, ama department of drugs, ama drug evaluations. 3rd ed. littleton, massachusetts: psg publishing co., inc., 1977., p. 663] **peer reviewed**

Study carried out in printing works in stuttgart revealed symptoms such as incipient or clearly defined asthma, catarrh &...irritation of nasal mucous, sinus, throat, respiratory tract and bronchus. cases of fainting fits among women exposed to gum arabic have been recorded. [international labour office. encyclopedia of occupational health and safety. volumes i and ii. new york: mcgraw-hill book co., 1971., p. 629] **peer reviewed**

Examination of 37 printing workers revealed 13 cases of marked dyspnea, soon after exertion. a distinguishing feature was that the trouble appeared shortly after arrival at work and did not occur on non-working days. [international labour office. encyclopedia of occupational health and safety. volumes i and ii. new york: mcgraw-hill book co., 1971., p. 629] **peer reviewed**

Examination of 37 printing workers...soon after exertion. in 20 of the 37 printers, there were well-defined radiological findings in...lungs, with occasional chronic bronchitis, and pulmonary congestion. [international labour office. encyclopedia of occupational health and safety. volumes i and ii. new york: mcgraw-hill book co., 1971., p. 629] **peer reviewed**

Examination of 37 printing workers revealed...dyspnea soon after exertion. in 9 cases vital capacity was appreciably reduced, even in workers who were otherwise apparently without subjective disturbances. intracutaneous injection of 1% gum arabic soln produced positive reaction in 16 of 37 workers. [international labour office. encyclopedia of occupational health and safety. volumes i and ii. new york: mcgraw-hill book co., 1971., p. 629] **peer reviewed**

An attempt at passive transmission using the serum from a patient suffering from printer's asthma has proved positive, and 11 employees of a printworks who had never had any ill effects also showed positive reactions. further studies have shown that sensitization occurred in about 50% of workers. [international labour office. encyclopedia of occupational health and safety. volumes i and ii. new york: mcgraw-hill book co., 1971., p. 629] **peer reviewed**

As taken from HSDB, 2002.

6.2. Cardiovascular system

Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies was asked to provide a scientific opinion on a list of health claims pursuant to Article 13 of Regulation (EC) No 1924/2006. This opinion addresses the scientific substantiation of health claims in relation to acacia gum (gum arabic) and reduction of post-prandial glycaemic response and maintenance of normal blood glucose concentrations. The scientific substantiation is based on the information provided by the Member States in the consolidated list of Article 13 health claims and references that EFSA has received from Member States or directly from stakeholders. The food constituent that is the subject of the health claim is acacia gum. The Panel considers that acacia gum is sufficiently characterized. (EFSA, 2010b)

Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies was asked to provide a scientific opinion on a list of health claims pursuant to Article 13 of Regulation 1924/2006. This opinion addresses the scientific substantiation of health claims in relation to acacia gum (gum Arabic) and maintenance of normal blood cholesterol concentrations. The scientific substantiation is based on the information provided by the Member States in the consolidated list of Article 13 health claims and references that EFSA has received from Member States or directly from stakeholders. The food constituent that is the subject of the health claim is acacia gum (gum Arabic). Acacia gum is a water-soluble type of fiber non-digestible in the human small intestine. The Panel considers that acacia gum is sufficiently characterized. The claimed effect is “cholesterol”. In the context of the proposed wordings the Panel assumes that the claimed effect relates to the maintenance of normal blood cholesterol concentrations. The Panel considers that maintaining normal blood cholesterol concentrations is beneficial to human health. Five intervention studies investigating the effects of acacia gum on serum lipids in humans have been provided. In weighing the evidence the Panel took into account that acacia gum has a relatively low viscosity, and that its effects on blood cholesterol have been weak or non-detectable in the small, and often uncontrolled, clinical trials presented despite the relatively high doses used. On the basis of the data available, the Panel concludes that a cause and effect relationship has not been established between the consumption of acacia gum and maintenance of normal blood cholesterol concentrations. (EFSA, 2009)

Gum arabic (GA) is shown to conform to the definitions of dietary fibre, now finally adopted by the European Union and Codex Alimentarius. A non-starch polysaccharide, GA is not digested in the intestine but is fermented in the colon to give short-chain fatty acids, leading to a wide range of potential health benefits. An obstacle to regulatory approval of such health applications could be the wide natural variability of commercial gum arabic which has been demonstrated to change its molecular parameters and functional properties. For this reason, a well characterized and specific gum arabic (Acacia (sen) SUPERGUM™) has been produced, which has guaranteed structural reproducibility. We report here on the studies in vivo and in vitro with this material, which show its compatibility in the diet of patients suffering with diabetes mellitus and reduction in systolic blood pressure, which may translate into improved cardiovascular outcome and a reduction in the progression of renal disease (Philips A. O. et al., 2011)

“Acacia arabica and Moringa oleifera are credited with a number of medicinal properties. Traditionally gum of Acacia plant is used in the treatment of skin disorders to soothe skin rashes,

soreness, inflammation and burns while Moringa seed extracts are known to have antibacterial activity. In the present study the potential of the polymeric component of aqueous extracts of gum acacia (GA) and the seeds of M. oleifera (MSP) in wound management was evaluated. The results revealed that both biopolymers were hemostatic and hasten blood coagulation. They showed shortening of activated partial thromboplastin time and prothrombin time....They were biodegradable and exhibited water absorption capacity in the range of 415 to 935%. The hemostatic character coupled to these properties envisions their potential in preparation of dressings for bleeding and profusely exuding wounds. The biopolymers have been further analysed for their composition by Gas chromatography." As taken from Bhatnagar M et al. 2013. Indian. J. Exp. Biol. 51(10), 804-10. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24266104

"White rice porridge and mixed grain porridge, which are often consumed in many countries, were used as two models to evaluate the effects of gum arabic on glucose levels and microbial short-chain fatty acids (SCFA). Gum arabic was incorporated into the two porridges individually. Apparent viscosity of the two porridges was significantly increased, and their glucose productions during gastrointestinal digestion were notably lowered ($p < 0.05$). Diffused glucose amount was significantly decreased after gum arabic addition ($p < 0.05$). Furthermore, blood glucose rise after oral administration of porridges in mice was considerably lowered after fortified with gum arabic ($p < 0.05$). Microbial SCFA production during in vitro fermentation of porridges was significantly increased after gum arabicaddition, which may also have beneficial effects on reducing postprandial glycemic response. Therefore, gum arabicmay be a helpful ingredient, which could be added in porridges to have benefits for the reduction of postprandial glycemic response." As taken from Hu JL et al. 2014. J. Agric. Food Chem. 62(27), 6408-16. PubMed, 2015 available at http://www.ncbi.nlm.nih.gov/pubmed/24941348

6.3. Nervous system

No data available to us at this time.

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

Acacia can be digested by rats to an extent of 71%; guinea pigs and rabbits also seem to use it for energy, as do humans to a certain extent. ... Acacia may actually elevate serum or tissue cholesterol levels in rats. [Leung, A.Y., Foster, S. Encyclopedia of Common Natural Ingredients Used in Food, Drugs, and Cosmetics. New York, NY. John Wiley & Sons, Inc. 1996., p. 5] **PEER REVIEWED**

As taken from HSDB, 2002.

Gum arabic (GA) modifies paracellular water and electrolyte transport in the small intestine (Abstract).

Previous experiments have shown that a soluble fiber, gum arabic (GA), enhances water, electrolyte, and glucose absorption in animal models of diarrhea. The mechanisms implicated in this effect have not been fully elucidated. This study examined the possibility that paracellular transport is modulated by luminal GA, resulting in an enhanced rate of absorption in the small intestine. This hypothesis was tested by 3-hr jejunal perfusions on anesthetized rats with solutions containing 140 mM NaCl, 5 mM KCl, and 2 microCi/liter (74 kBq) 3H2O, with either 2.5 g/liter GA [+GA] or in its absence [CTL], and one of the following agents, capable of altering paracellular transport: chenodeoxycholic acid at 0.5 mM (CDC), 2,4,6-triaminopyrimidine (TAP) at 20 mM, and protamine at 100 mg/liter (PTM). Sodium, potassium, net water, and unidirectional water movement were measured. The addition of GA increased sodium absorption in perfusions with CDC, TAP, or PTM only. Similar effects by GA on net water absorption rates were obtained in tissues permeabilized with CDC and PTM; however, GA added to TAP did not normalize the reduction

caused by TAP. Although PTM did not alter net water absorption, addition of GA to perfusates with PTM enhanced absorption values above those of CTL. GA reversed the strong negative effects of CDC on potassium absorption but was ineffective in this regard with TAP and PTM. The data obtained with those reagents that affect paracellular transport and the histological evidence support the view that GA promotes net absorption by this route in the small intestine of normal rats. As taken from Rehman KU et al. (2003).

Effects of gum arabic (Acacia senegal) on water and electrolyte balance in healthy mice (Abstract).

OBJECTIVE: Gum arabic (GA) is a dietary fiber derived from the dried exudates of Acacia senegal. It is widely used in both the pharmaceutical and food industries as an emulsifier and stabilizer. It is also used in the traditional treatment of patients with chronic kidney disease in Middle Eastern countries. However, the effects of GA on renal function remain ill-defined.

DESIGN: We explored the effects of GA on the water and electrolyte balance of healthy wild-type 129S1/SvImJ mice (n = 18). Feces and urine were collected in metabolic cages before and after 3 or 14 days of treatment with 10% GA in drinking water.

RESULTS: The GA solutions contained particularly high concentrations of Ca2+, Mg2+, and K+. Because of enhanced uptake, treatment with GA significantly increased both the intestinal and renal excretion of Mg2+ and Ca(2+). The latter was accompanied by decreased urinary excretion of inorganic phosphate and decreased plasma concentrations of 1,25-dihydroxy vitamin D. Moreover, GA significantly increased fecal weight and Na+ excretion. Gum arabic increased 24-h creatinine clearance (from 283 +/- 35 to 382 +/- 40 muL/min [SEM]) and urinary antidiuretic hormone excretion, and decreased daily urine output (from 1.8 +/- 0.2 to 1.2 +/- 0.1 mL/24 h) as well as the urinary excretion of Na(+) (from 226 +/- 22 to 196 +/- 19 mumol/24 h). In conclusion, treatment with GA resulted in moderate but significant increases of creatinine clearance and altered electrolyte excretion, i.e., effects favorable in renal insufficiency. Nasir O et al. (2008).

Stimulation of mouse dendritic cells by Gum Arabic (Abstract)

Gum Arabic (GA), a nonabsorbable nutrient manufactured from the exudate of Acacia senegal, is composed of a complex polysaccharide. GA is used by the pharmaceutical and food industry as an emulsifier but may, at an appropriate dosage, modify intestinal transport. Dendritic cells (DCs) can protrude between epithelial cells and sense the composition of the lumen. As DCs are stimulated by bacterial polysaccharides, we hypothesized that GA may similarly stimulate DCs. To test that hypothesis, mouse DCs were treated with either LPS or GA and expression of maturation markers, phagocytotic activity, cytokine production and ability to stimulate CD4(+) T cells in allogenic mixed leukocyte reaction (allo-MLR) was analyzed. As a result both LPS and GA increased the percentage of CD11c(+)CD86(+), CD11c(+)MHCII(+), CD11c(+)CD40(+), CD54-expressing DCs and decreased their phagocytic activity. Both LPS and GA stimulated the production of IL-6, IL-10, IL12p70 and TNFalpha in a p38- and/or ERK-dependent manner. GA treatment led to an enhanced IL-10 secretion, whereas LPS was more effective on IL-6 and IL-12p70 production. Both LPS- and GA-stimulated DCs enhanced CD4(+) T cell proliferation but the profile of cytokines produced in allo-MLR was different. High levels of IL-10 and IL-6 were observed in the presence of GA-treated DCs, whereas IFN-gamma and IL-12p70 production was similar with LPS- or GA-treated DCs. LPS upregulated p38 and transiently ERK1/2, while GA led to more sustained activation of ERK1/2, only. In conclusion, the observations reveal a powerful immunomodulatory effect of GA. Xuan NT et al. (2010).

Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies was asked to provide a scientific opinion on a list of health claims pursuant to Article 13 of Regulation (EC) No 1924/2006. This opinion addresses the scientific substantiation of health claims in relation to acacia gum (gum Arabic) and decreasing potentially pathogenic gastro-intestinal microorganisms, changes in short chain fatty acid (SCFA) production and pH in the gastro-intestinal tract, changes in bowel function, reduction of gastro-intestinal discomfort, maintenance of faecal nitrogen content and/or normal blood urea concentrations, and maintenance of normal blood LDL-

cholesterol concentrations. The scientific substantiation is based on the information provided by the Member States in the consolidated list of Article 13 health claims and references that EFSA has received from Member States or directly from stakeholders. The food constituent that is the subject of the health claims is acacia gum (gum Arabic). The Panel considers that acacia gum is sufficiently characterized (EFSA, 2011).

“.... 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-bring and health."....Other isolated carbohydrates and carbohydrate-containing foods, including....acacia gum... also have prebiotic effects.” As taken from Slavin J et al. 2013. Nutrients 5(4), 1417-35. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23609775

“Gum Arabic (GA) is known for its proabsorbent activity in normal intestine as well as in animal models of diarrhea. The aim of the study was to find the effect of GA on intestinal transport of water and possible route of absorption in frog everted gut sacs. D-Mannitol was used as a marker of paracellular transport to find the route of absorption. Everted gut sacs (n = 4,5) were placed in Ringer containing GA (2.5 g/L) with or without D-Mannitol (0.5 g/L), incubated for 1 hour and analysed for change in weights of the sacs and D-Mannitol uptake. There was significant increase in uptake of water and D-Mannitol in the presence of GA compared to controls ($P < 0.05$). Gum Arabicimproves water uptake by the intestinal mucosa, possibly by opening the paracellular pathways.” As taken from Pai MK et al. 2013. Indian J. Physiol. Pharmacol. 57(2), 195-8. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24617171

“Gum arabic (GA), a water-soluble dietary fiber rich in Ca(2+), Mg(2+) and K(+), is used in Middle Eastern countries for the treatment of patients with chronic kidney disease. Recent animal experiments shed some light into mechanisms involved in the therapeutic action of GA. According to experiments in healthy mice, GA treatment increases creatinine clearance, enhances renal excretion of ADH, Mg(2+) and Ca(2+), decreases plasma phosphate concentration as well as urinary excretion of phosphate and Na(+). In diabetic mice GA treatment increases urinary Ca(2+) excretion, and decreases plasma phosphate concentration, plasma urea concentration, urinary flow rate, natriuresis, phosphaturia, glucosuria, proteinuria as well as blood pressure. Extrarenal effects of GA treatment in mice include decreased expression of intestinal Na(+) coupled glucose carrier SGLT1 with subsequent delay of electrogenic intestinal glucose transport, glucose-induced hyperglycemia, hyperinsulinemia and body weight gain. GA treatment decreases colonic transcription of the angiogenetic factors angiogenin 1, angiogenin 3 and angiogenin 4, of CD38 antigen, aquaporin4, interleukin18, vav-3-oncogene, y(+)-amino acid-transporter, sulfatase1, ubiquitinD and chemokine ligand5. Moreover, GA treatment decreases angiogenin and ß-catenin protein expression....GA treatment further favourably influences the course of murine malaria. The effects of GA treatment on plasma phosphate concentration, blood pressure and proteinuria may prove beneficial in chronic renal failure and diabetic nephropathy. The effect of GA on intestinal glucose transport may be useful in the prophylaxis and treatment of obesity and diabetes, the effect of GA on angiogenin and ß-catenin expression could be exploited for the prophylaxis against colon carcinoma, the effects of GA on angiogenin expression and dendritic cells may be useful in the treatment of inflammatory disease and malaria.” As taken from Nasir O. 2013. Kidney Blood Press. Res. 37(4-5), 269-79. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24022265

“BACKGROUND/AIM: Chronic kidney disease (CKD) is associated with increased occurrence of cardiovascular system dysfunction. Previous studies have revealed a number of alterations in the kidneys and heart during CKD. However, unbiased quantitative studies on these structures in this disease have so far not been addressed. MATERIALS AND METHODS: We induced CKD in rats by feeding adenine (0.75% (w/w), four weeks) and using unbiased stereological methods, investigated the effect of the ensuing CKD on the kidneys and left ventricular structure. Since gum acacia (GA) has previously been shown to ameliorate the severity of CKD in humans and rodents,

we investigated the effect of giving GA (15% (w/v) in the drinking water concomitantly with adenine) on the kidneys and left ventricular structure using the above model. RESULTS: The CKD was confirmed by standard biochemical indices in plasma and urine and by accumulation of the uremic toxin indoxyl sulfate. Additionally, it increased blood pressure. In rats with CKD absolute volume of left ventricle was significantly increased, and the volume density and absolute volume of myocardial capillaries were decreased, whilst the same parameters of myocardium and interstitial tissue were increased. Renal morphometry demonstrated significant increase in kidney volume and interstitial tissue in adenine- treated rats. Similarly, glomerular Bowman's capsule was significantly thickened. The myocardial and renal changes were significantly mitigated by GA treatment. CONCLUSIONS: These results add to our existing knowledge of the pathophysiology of adenine - CKD and provides plausible histopathological and morphometric evidence for the usefulness of GA in CKD." As taken from Ali BH et al. 2014. Cell. Physiol. Biochem. 34(3), 818-28. PubMed, 2015 available at http://www.ncbi.nlm.nih.gov/pubmed/25171124

"OBJECTIVE: This study was conducted in order to investigate the effects of adenine-induced chronic kidney disease (CKD) on renal blood flow and biochemical changes in rats, and to assess the effect of treatment with gum acacia (GA) thereon. MATERIALS AND METHODS: CKD was induced by feeding rats with adenine (0.25% w/w, five weeks). Concomitantly, some of these rats were also given gum acacia (GA) (15% w/v in the drinking water). Before animals were sacrificed, changes in renal blood flow (RBF) were monitored in anaesthetized rat preparations. Several biochemical and histological renal function tests were also conducted. RESULTS: Adenine-induced CKD significantly impaired the vasopressor actions of acetylcholine, sodium nitroprusside and phenylephrine and concomitant treatment with GA abated these responses. Additionally, plasma concentrations of urea, creatinine, uric acid, indoxyl sulfate, nitrite and nitrate and urinary excretion of protein were all significantly increased by adenine. GA significantly mitigated the severity of adenine-induced changes. CONCLUSIONS: Adenine-induced CKD in rats significantly impaired renal vascular responses to acetylcholine, sodium nitroprusside and phenylephrine and this was mitigated by treatment with GA. This provides another experimental evidence for the usefulness of GA in the amelioration of CKD." As taken from Al Suleimani YM et al. 2015. Eur. Rev. Med. Pharmacol. Sci. 19(3), 498-506. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/25720725

Gum arabic-coated radioactive gold nanoparticles (GA-(198)AuNPs) offer several advantages over traditional brachytherapy in the treatment of prostate cancer, including homogenous dose distribution and higher dose-rate irradiation. Our objective was to determine the short-term safety profile of GA-(198)AuNPs injected intralesionally. We proposed that a single treatment of GA-(198)AuNPs would be safe with minimal-to-no evidence of systemic or local toxicity. METHODS:

Nine dogs with spontaneously occurring prostatic cancer were treated. Injections were performed with ultrasound or computerized tomography guidance. Complete blood counts, chemistry panels, and urinalyses were performed at weekly intervals for 1 month and imaging was repeated 4 weeks postinjection. Planar scintigraphic images were obtained within 30 minutes of injection.RESULTS:

No statistically significant difference was found in any hematologic or biochemical parameter studied, nor was any evidence of tumor swelling or abscessation found in eight dogs with repeat imaging; one dog died secondary to urethral obstruction 12 days following injection. At 30 minutes postinjection, an average of 53% of injected dose in seven dogs was retained in the prostate, with loss of remaining activity in the bladder and urethra; no systemic uptake was detected. CONCLUSION: GA-(198)AuNP therapy had no short-term toxicity in the treatment of prostatic cancer. While therapeutic agent was found in the prostate immediately following injection, some loss of agent was detected in the bladder and urethra. Localization of radioactivity within the prostate was lower than anticipated and likely due to normal vestigial prostatic ducts. Therefore, further study of retention, dosimetry, long-term toxicity, and efficacy of this treatment is warranted prior to Phase I trials in men. Axiak-Bechtel SM et al. (2014)

“Arabic gum (AG) has antioxidant and anti-inflammatory properties. However, the effect of AG in ureteric obstruction (UO) has not been investigated yet. Male rats underwent reversible left unilateral UO (UUO) for 72 h. Group AG-1 (n = 12) received AG 15 g/kg/day dissolved in drinking water starting seven days before and continuing throughout the period of the UUO, whereas group Vx-1 (n = 8) had only water. Group AG-2 (n = 12) and Vx-2 (n = 8) had similar protocols as AG-1 and Vx-1, respectively, but underwent terminal experiments to measure renal functions, six days post-UUO reversal. Arabic gum significantly attenuated the UUO-induced increase in the tissue level of malonedialdehyde and superoxide dismutase and the rise in the gene expression of TNF-α, TGF-β1, and p53 in AG-1 compared to Vx-1. It also attenuated the severity of tubular dilatation. However, AG did not affect the alterations in the renal blood flow or glomerular filtration rate. The fractional sodium excretion was lower in AG-2 but did not reach statistical significance (0.40 ± 0.11 vs 0.74 ± 0.12, p = 0.07). AG attenuated the UUO-induced rise in oxidative stress markers and proinflammatory and profibrotic cytokines and the degree of renal tubular dilatation, indicating a protective effect in obstructive nephropathy.” As taken from Hammad FT et al. 2019. Biomolecules 9(1), E25. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30641998

“Background Sickle cell anemia (SCA) is a hereditary chronic hemolytic anemia with several clinical consequences. Intravascular sickling of red blood cells leads to multi-organ dysfunction. Moreover, several biochemical abnormalities have been associated with SCA. Gum arabic (GA) is an edible dried gummy exudate obtained from Acacia Senegal tree. GA showed antioxidant and cytoprotective activities and demonstrated protection against hepatic, renal, and cardiac toxicities in experimental rats. We hypothesized that regular intake of GA improves renal and liver functions in patients with SCA. Methods Forty-seven patients (5–42 yr) carrying hemoglobin SS were recruited. The patients received 30 g/day GA for 12 weeks. Blood samples were collected before administering GA and then after 4, 8, and 12 weeks. Liver enzymes, total protein, albumin, electrolytes, urea, creatinine, and uric acid were determined in the serum. The study was approved by the Al Neelain University Institutional Review Board and Research Ethics Committee Ministry of Health. The trial was registered at ClinicalTrials.gov (identifier: NCT02467257). Results GA significantly decreased direct bilirubin level [statistical significance (P-value)=0.04]. It also significantly decreased serum alanine transaminase level after 4 weeks, which was sustained till the 8th week. GA, however, had no effect on serum aspartate transaminase level. In terms of renal function, GA decreased serum urea level but the effect was not sustained after the first month. Conclusion GA may alter the disease severity in SCA as demonstrated by its ability to decrease direct bilirubin and urea levels in the serum.” As taken from Kaddam LAG et al. 2019. Blood Res. 54(1), 31-37. Available at https://synapse.koreamed.org/search.php?where=aview&id=10.5045/br.2019.54.1.31&code=3072BR&vmode=FULL

“Chronic kidney disease (CKD) may be fatal for its victims and is an important long-term public health problem. The complicated medical procedures and diet restrictions to which patients with CKD are subjected alter the gut microbiome in an adverse manner, favoring over-accumulation of proteolytic bacteria that produce ammonia and other toxic substances. The present study aimed to investigate the effect of GA on 1) the composition of the gut microbiome and 2) on plasma levels of short-chain fatty acids. Male Wister rats were divided into four groups (six each) and treated for 4 weeks based on the following: control, dietary adenine (0.75%, w/w) to induce CKD, GA in the drinking water (15%, w/v), and both adenine and GA. At the end of the treatment period, plasma, urine, and fecal samples were collected for determination of several biochemical indicators of renal function and plasma levels of short-chain fatty acids (SCFAs) as well as characterization of the gut microbiome. Dietary adenine induced the typical signs of CKD, i.e., loss of body weight and impairment of renal function, while GA alleviated these effects. The intestine of the rats with CKD contained an elevated abundance of pathogenic Proteobacteria, Actinobacteria, and Verrucomicrobia but lowered proportions of Lactobacillaceae belonging to the Firmicutes phylum. Plasma levels of propionate and butyrate were lowered by dietary adenine and restored by GA. A negative association (Spearman's p-value ≤ 0.01, r ≤ 0.5) was observed between Firmicutes and

plasma creatinine, urea, urine N-acetyl-beta-D-glucosaminidase (NAG) and albumin. Phylum Proteobacteria on the other hand was positively associated with these markers while Phylum Bacteroidetes was positively associated with plasma SCFAs. In conclusion, the adverse changes in the composition of the gut microbiome, plasma levels of SCFAs, and biochemical indicators of renal function observed in the rats with CKD induced by dietary adenine were mitigated by GA. These findings are indicative of a link between uremia and the composition of the microbiome in connection with this disease. Dietary administration of GA to patients with CKD may improve their renal function via modulating the composition of their microbiome-a finding that certainly warrants further investigation." As taken from Al-Asmakh M et al. 2020. Front. Pharmacol. 11, 569402. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33628167/

"Aim of the work: the use of ionizing radiation exposure increases, oxidative stress especially for cancer patients. Therefore, there is a critical need to develop antioxidants that prevent oxidative stress damage. Gum Arabic is an antioxidant and anti-inflammatory mediator. This study aimed to assess the possible radio protective effect of gum Arabic (Acacia senegal) against gamma irradiation-induced injury on the rat kidney. Materials and Methods: four groups of male Albino rats (each of 12 rats) received normal saline (G1), dose 5Gy of gamma irradiation (G2), orally administered with 25 mg/kg gum Arabic for 3 weeks (G3) and gamma irradiation plus oral gum Arabic administration for 7 days before and 21 days after irradiation (G4). Results: in the gamma irradiated group, histopathological and ultrastructural examinations of the rat kidney cortical tissue revealed signs of degeneration to the cortical renal tubules and glomeruli. In contrast, gum Arabic treatment alleviated most of the damaging effects of gamma radiation. Conclusion: oral administration of gum Arabic could ameliorate adverse gamma radiation-induced effects that might be attributed to its antioxidative and free radical scavenging effects." As taken from Kandeal HAM et al. 2021. The Egyptian Journal of Hospital Medicine 82(2), 256-269. Available at https://ejhm.journals.ekb.eg/article_143886.html

"Arabic gum (AG) is a natural branched-chain multifunctional hydrocolloid with a highly neutral or slightly acidic, arabinogalactan protein complex containing calcium, magnesium, and potassium. Arabic gum is a dried exudate obtained from the stem and branches of Acacia trees. AG has an anti-inflammatory and antioxidant effect. This study aimed to examine the possible anti-inflammatory and antioxidant effect of AG against the damage induced by alloxan injection on renal tissue of adult male rats using histological and histochemical studies. The current experiment was carried out on 48 adult male Albino rats. Rats were randomly and equally categorized into four groups 12 rats in each group as follows: 1) Control group (C): rats have left without treatment; 2) Diabetic groups (D): rats were injected with 150 mg/kg body weight of alloxan and left for 21 days ; 3) Arabic gum groups (G): rats were orally administrated (25 mg/kg body weight/day) for 21 days and 4) D+G groups: rats were injected with alloxan and treated with 25 mg/kg body weight/day for 21 days. The experimental rats were sacrificed after 7 and 21 days post-treatment. Examination of renal tissue of rats seven and twenty-one days post-alloxan injection revealed many histological and histochemical changes. Highly increased collagen fibres were demonstrated after seven and twenty-one days post alloxan injection. Also, alloxan injection significantly decreased PAS-positive materials, total protein content, and total DNA content, but it significantly increased amyloid β – protein content relative to the control group. While treatment with AG post-alloxan injection showed a trend toward lowering the incidence of renal tissue histological and histochemical changes induced by alloxan injection. According to the results obtained in the current study using AG as a natural agent showed a strong cytoprotective effect against the histological and histochemical changes due to its antioxidant effect." As taken from Ibrahim RM et al. 2020. Journal of Medical and Life Science 2(4), 69-90. Available at https://journals.ekb.eg/article_133543.html

"Context: Transforming growth factor-β1 (TGF-β1), endothelin-1 and angiotensin II are responsible for extracellular matrix accumulation within the kidney in diabetic nephropathy. Objective: This study evaluated the effect of adding Gum Arabic (GA) and insulin on serum glucose, renal function, TGF-β1, endothelin-1, and angiotensin II in rats with diabetic nephropathy. Methods: Sixty male Sprague-Dawley rats were divided into; normal, normal plus GA, diabetic rats (DM), DM plus

insulin, DM plus GA, and DM plus insulin plus GA groups. Levels of glucose and creatinine in serum, TGF-β1, angiotensin II, and endothelin-1 in renal homogenate and HbA1c were measured. Results: Serum creatinine, TGF-β1, angiotensin II, and endothelin-1 were increased in diabetic rats. GA decreased serum glucose, TGF-β1, angiotensin II, endothelin-1, and HbA1c in diabetic rats. GA and insulin decreased serum glucose, creatinine, TGF-β1, angiotensin II, endothelin-1, and HbA1c in diabetic rats. Conclusion: Co-administration of GA with insulin to rats with diabetic nephropathy improved the glycemic state, renal function, TGF-β1, endothelin-1, and angiotensin II." As taken from Mohammed ME et al. 2020. Arch. Physiol. Biochem. Epub ahead of print. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32574082/

"The present study was performed to evaluate the anti-ulcerogenic activity of Acacia senegal (Gum Arabic) against ethanol-induced gastric mucosal injury in rats. Thirty-six adult male albino rats were divided into 4 groups: group 1 served as a control; group 2 consisted of rats that received 15% of gum in drinking water for 2 weeks; group 3 comprised ulcerated animals administered 5 mL of ethanol/kg body weight by gavage; and group 4 consisted of rats received 15% of gum in drinking water for 2 weeks before ethanol administration. Superoxide dismutase (SOD) glutathione peroxidase (GPx), malondialdehyde (MDA), prostaglandin E2 (PGE2), tumor necrosis factor alpha (TNF-α), interleukin (IL)-B1), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, and albumin were assayed in addition to histological study. The results revealed that ethanol decreased SOD, GPx, and PGE2 in tissue and serum total protein and albumin, while increased MDA in tissue, serum TNF-α, IL-B1, PGE2, ALT, AST, and ALP. Histological findings showed less edema and leucocytes infiltration compared with ulcer group. Furthermore, gum administration elevated PGE2, SOD, and GPx and significantly reduced MDA, TNF-α, and IL-B2. In conclusion, Gum Arabic can enhance gastric protection and sustain the integrity of the gastric mucosa." As taken from Taha MS et al. 2020. Appl. Physiol. Nutr. Metab. 45(7), 731-736. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/31905015/

Ethnopharmacological relevance: Angelica roots are a significant source of traditional medicines for various cultures around the northern hemisphere, from indigenous communities in North America to Japan. Among its many applications, the roots are used to treat type 2 diabetes mellitus; however, this application is not mentioned often. Ethnopharmacological studies have reported the use of A. japonica var. hirsutiflora, A. furcijuga, A. shikokiana, and A. keiskei to treat diabetes symptoms, and further reports have demonstrated the three angelica roots, i.e., A. japonica var. hirsutiflora, A. reflexa, and A. dahurica, exhibit insulin secretagogue activity.
Aim of the study: This study aimed to phytochemically characterize and compare angelica roots monographed in the European Pharmacopeia 11th, isolate major plant metabolites, and assess extracts and isolates' capability to modulate pancreatic β-cell function.
Materials and methods: Root extracts of Angelica archangelica, Angelica dahurica, Angelica biserrata, and Angelica sinensis were phytochemically profiled using liquid chromatography method coupled with mass spectrometry. Based on this analysis, simple and furanocoumarins were isolated using chromatography techniques. Extracts (1.6-50 μg/mL) and isolated compounds (5-40 μmol/L) were studied for their ability to modulate insulin secretion in the rat insulinoma INS-1 pancreatic β-cell model. Insulin was quantified by the homogeneous time-resolved fluorescence method.
Results: Forty-one secondary metabolites, mostly coumarins, were identified in angelica root extracts. A. archangelica, A. dahurica, and A. biserrata root extracts at concentration of 12.5-50 μg/mL potentiated glucose-induced insulin secretion, which correlated with their high coumarin content. Subsequently, 23 coumarins were isolated from these roots and screened using the same protocol. Coumarins substituted with the isoprenyl group were found to be responsible for the extracts' insulinotropic effect.
Conclusions: Insulinotropic effects of three pharmacopeial angelica roots were found, the metabolite profiles and pharmacological activities of the roots were correlated, and key structures responsible for the modulation of pancreatic β-cell function were identified. These findings may have implications for the traditional use of angelica roots in treating diabetes. Active plant metabolites may also become lead structures in the search for new antidiabetic treatments.

Patyra, Andrzej et al. (2024) “Pharmacological and phytochemical insights on the pancreatic β-cell modulation by Angelica L. roots.” Journal of ethnopharmacology vol. 329 (2024): 118133. doi:10.1016/j.jep.2024.118133

7. Addiction

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

8. Burnt ingredient toxicity

Tobacco smoke condensates from cigarettes containing Gum Arabic, absolute 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 Gum arabic, absolute. Table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
Smoke chemistry	15000 2105	Baker et al., 2004c JTI NTM Study Report
In vitro genotoxicity	15000 2105	Baker et al., 2004c JTI NTM Study Report
In vitro cytotoxicity	15000 2105	Baker et al., 2004c JTI NTM Study Report
Inhalation study	15000	Baker et al., 2004c

JTI NTM Study Report(s)

9. Heated/vapor emissions toxicity

Aerosol from heated tobacco stick(s) containing acacia gum 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	3.56386	Labstat International Inc. (2023a) JTI Heated Tobacco Stick Study Report(s)
In vitro genotoxicity	3.56386	Labstat International Inc. (2023b) JTI Heated Tobacco Stick Study Report(s)
In vitro cytotoxicity	3.56386	Labstat International Inc. (2023b) JTI Heated Tobacco Stick Study Report(s)

10. Ecotoxicity

10.1. Environmental fate

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that gum arabic is of uncertain persistence in the environment.

Data accessed June 2017 on the OECD website

10.2. Aquatic toxicity

Record for gum arabic:

Spec. Sci. Name Spec. Common Name	Exp. Type Chem. Anal.	Media Type Loc	Resp. Site Obs. Dur. (Days)	Endpoint BCF	Trend Eff %	Effect Effect Meas.	Conc (Standardized) Appl. Rate	Stat. Signif. Sig. Level
Xenopus laevis African Clawed Frog	R U	FW LAB	12	NOEC	DEC >80-<100/	GRO GRRT/	F 50000 ug/L	ANOSIG 0.05
Xenopus laevis African Clawed Frog	R U	FW LAB	12	NR-ZERO	NEF 0	MOR MORT	F 50000 ug/L	
Xenopus laevis African Clawed Frog	R U	FW LAB	BL 12		DEC	GEN MNUC/	F 50000 ug/L	

As taken from the US EPA ECOTOX database.

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that gum arabic is not inherently toxic to aquatic organisms and is of low ecotoxicological concern.

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

“BACKGROUND: Diabetes mellitus is a chronic metabolic disease with life-threatening complications. Despite the enormous progress in conventional medicine and pharmaceutical industry, herbal-based medicines are still a common practice for the treatment of diabetes. This study evaluated ethanolic and aqueous extracts of selected Sudanese plants that are traditionally used to treat diabetes. METHODS: Extraction was carried out according to method described by Sukhdev et. al. and the extracts were tested for their glycogen phosphorylase inhibition, Brine shrimp lethality and antioxidant activity using (DPPH) radical scavenging activity and iron chelating activity. Extracts prepared from the leaves of Ambrosia maritima, fruits of Foeniculum vulgare and Ammi visnaga, exudates of Acacia Senegal, and seeds of Sesamum indicum and Nigella sativa. RESULTS: Nigella sativa ethanolic extract showed no toxicity on Brine shrimp Lethality Test, while

its aqueous extract was toxic. All other extracts were highly toxic and ethanolic extracts of Foeniculum vulgare exhibited the highest toxicity. All plant extracts with exception of Acacia senegal revealed significant antioxidant activity in DPPH free radical scavenging assay. CONCLUSIONS: These results highly agree with the ethnobotanical uses of these plants as antidiabetic. This study endorses further studies on plants investigated, to determine their potential for type 2 diabetes management. Moreover isolation and identification of active compounds are highly recommended." As taken from Hilmi Y et al. 2014. BMC Complement. Altern. Med. 14, 149. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/24885334

10.3. Sediment toxicity

No data available to us at this time.

10.4. Terrestrial toxicity

No data available to us at this time.

10.5. All other relevant types of ecotoxicity

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

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

11. References

- Abubakar AL et al. (2018). Antimicrobial activities of Acacia nilotica, Ziziphus jujube Linn and Lawsonia inermis. Nigerian Journal of Basic and Applied Science 26(2), 01-08. DOI: 10.4314/njbas.v26i2.1. Available at https://www.ajol.info/index.php/njbas/article/view/184782
- AICIS (2017). Australian Government Department of Health. Australian Industrial Chemicals Introduction Scheme. Inventory Multi-Tiered Assessment and Prioritisation (IMAP) Tier I. Health record for gum arabic (CAS RN 9000-01-5). Dated 10 March 2017. Available at: https://services.industrialchemicals.gov.au/search-assessments/
- Al Suleimani YM et al.(2015). Influence of treatment with gum acacia on renal vascular responses in a rat model of chronic kidney disease. Eur. Rev. Med. Pharmacol. Sci. 19(3), 498-506. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/25720725
- Al-Asmakh M et al. (2020).The Effects of Gum Acacia on the Composition of the Gut Microbiome and Plasma Levels of Short-Chain Fatty Acids in a Rat Model of Chronic Kidney Disease.Front. Pharmacol.11, 569402. DOI: 10.3389/fphar.2020.569402. PubMed, 2021 available at
- Al-Doaiss AA and Al-Shehri MA (2020). Protective effect of gum Arabic/insulin against histological changes in testes of diabetic rats.Int. J. Morphol., 38(2), 340-347.Available at https://bit.ly/2VMT6CC
- Ali BH et al. (2014). Renal and myocardial histopathology and morphometry in rats with adenine - induced chronic renal failure: influence of gum acacia. Cell. Physiol. Biochem. 34(3), 818-28. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/25171124
- Ali BH et al. (2015). High-mobility group box-1 protein in adenine-induced chronic renal failure and the influence of gum arabic thereon. Physiol. Res. 64(1), 147-51. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/25194125
- Axiak-Bechtel SM et al. (2014). Gum arabic-coated radioactive gold nanoparticles cause no short-term local or systemic toxicity in the clinically relevant canine model of prostate cancer. Int J Nanomedicine. 9, 5001-5011. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25378926

- Azouz RA and Hassanen EI (2020). Modulating Effect of Gum Arabic on Cisplatin-induced Testicular Damage in Albino Wister Rats. Rev. Bras. Farmacogn. 30, 90-98. DOI: 10.1007/s43450-020-00015-7. Available at https://link.springer.com/article/10.1007/s43450-020-00015-7
- Bagri P and Jain SK (2019). Assessment of acetamiprid-induced genotoxic effects in bone marrow cells of Swiss albino male mice.Drug Chem.Toxicol. 42(4), 357–363.DOI:10.1080/01480545.2018.1429461. PubMed, 2020 available at
- Baien SH et al. (2020). Antimicrobial and Immunomodulatory Effect of Gum Arabic on Human and Bovine Granulocytes Against Staphylococcus aureus and Escherichia coli. Front. Immunol. 10, 3119. DOI:10.3389/fimmu.2019.03119. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/32082302/
- Baker RR and Bishop LJ. (2005) the pyrolysis of non-volatile tobacco ingredients using a system that stimulates cigarette combustion conditions.J. Anal.Appl. Pyrolysis 74, 145–170.
- Baker RR et al. (2004a). The effect of tobacco ingredients on smoke chemistry. Part I: Flavourings and additives. Food and Chemical Toxicology 42s: S3-S37.
- Baker RR et al. (2004c). An overview of the effects of tobacco ingredients on smoke chemistry and toxicity.Food and Chemical Toxicology 42s: S53-S83.
- Bhatnagar M et al. (2013).Hemostatic, antibacterial biopolymers from Acacia arabica (Lam.) Willd. and Moringa oleifera (Lam.) as potential wound dressing materials.Indian. J. Exp. Biol. 51(10), 804-10. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24266104
- Bicho A, Roque AC, Cardoso AS, Domingos P, Batalha IL. (2010) “In vitro studies with mammalian cell lines and gum arabic-coated magnetic nanoparticles” J Mol Recognit; 23(6):536-42. http://www.ncbi.nlm.nih.gov/pubmed/21031476
- Binjumah M et al. (2018). Effects of the perinatal exposure of Gum Arabic on the development, behavior and biochemical parameters of mice offspring. Saudi J. Biol. Sci. 25(7), 1332-1338. DOI: 10.1016/j.sjbs.2016.04.008. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30505178
- Bovo F et al. (2016). Modulating Effects of Arabinogalactans from Plant Gum Exudates on Human Complement System. Scand. J. Immunol. 83(5), 314-20. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26972106
- Braga VNL et al. (2019). Gum arabic and red propolis protecteting colorectal preneoplastic lesions in a rat model of azoxymethane. Acta Cir. Bras. 34(2), e201900207. DOI: 10.1590/s0102-8650201900207. Available at: https://pubmed.ncbi.nlm.nih.gov/30843940/
- CCRIS (1995). Record for gum arabic. Last revision date 2 October 1995 (records no longer being updated after 2011).Available at https://www.toxinfo.io/
- Chandra Shekar BR et al.(2018). Antimicrobial efficacy of Acacia nilotica, Murraya koenigii L. Sprengel, Eucalyptus hybrid, Psidium guajava extracts and their combinations on Fusobacterium nucleatum and Porphyromonas gingivalis. Indian J. Dent. Res. 29(5), 641-645. DOI: 10.4103/ijdr.IJDR_52_17. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30409946
- CIR (2005). Final Report of 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. Cosmetic Ingredient Review Panel. International Journal of Toxicology 24(Suppl. 3), 75–118. Available at: http://ijt.sagepub.com/content/24/3_suppl/75
- Collins T.F.X. et al.(1987) Study of the teratogenic potential of gum arabic. Food and Chemical Toxicology, 25(11): 815–821 available at: http://www.sciencedirect.com/science/article/pii/0278691587902596
- CosIng (undated) Cosmetic substances and ingredients database. Available at https://ec.europa.eu/growth/tools-databases/cosing/

- CPID (undated). Consumer Product Information Database. Record for gum arabic (Acacia) (CAS RN 9000-01-5).Available at
- Daffalla HM (2018). Phytochemical, larvicidal and antimicrobial activities of gum Arabic extract. Walailak Journal of Science and Technology (WJST) 17. DOI: 10.14456/vol17iss5pp%p. Available at: http://wjst.wu.ac.th/index.php/wjst/article/view/5540
- Docea AO et al. (2018). Six months exposure to a real life mixture of 13 chemicals' below individual NOAELs induced non monotonic sex-dependent biochemical and redox status changes in rats. Food Chem. Toxicol. 115, 470-481. DOI: 10.1016/j.fct.2018.03.052. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29621577
- Doi Y. et al., (2006) “A ninety-day oral toxicity study of a new type of processed gum arabic, from Acacia tree (Acacia senegal) exudates, in F344 rats” Food and Chemical Toxicology; 44(4): 560–566.Available at: http://www.sciencedirect.com/science/article/pii/S0278691505003078
- ECHA (2023). European Chemicals Agency. Annex VI to the CLP Regulation. ATP 18. Applicable as of 1 December 2023. Available at: https://echa.europa.eu/information-on-chemicals/annex-vi-to-clp
- ECHA (undated). European Chemicals Agency. Information on Chemicals. Available at: https://echa.europa.eu/pl/information-on-chemicals/
- EFSA (2009). Scientific Opinion on the substantiation of health claims related to acacia gum and maintenance of normal blood cholesterol concentrations (ID 1976) pursuant to Article 13(1) of Regulation (EC) No 1924/2006 EFSA Journal 7(9), 1251.Available at: http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2009.1251/epdf
- EFSA (2010a). Scientific Opinion on the use of Gum Acacia modified with Octenyl Succinic Anhydride (OSA) as a food additive. EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS). EFSA Journal 8(3), 1539. Available at: http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2010.1539/epdf
- EFSA (2010b). Scientific Opinion on the substantiation of health claims related to acacia gum (gum arabic) and reduction of post-prandial glycaemic responses (ID 842, 1977) and maintenance of normal blood glucose concentrations (ID 842, 1977) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). EFSA Journal 8(2), 1475.Available at: http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2010.1475/epdf
- EFSA (2011). Scientific Opinion on the substantiation of health claims related to acacia gum (gum Arabic) and decreasing potentially pathogenic gastro-intestinal microorganisms (ID 758), changes in short chain fatty acid (SCFA) production and pH in the gastro-intestinal tract (ID 759), changes in bowel function (ID 759), reduction of gastro-intestinal discomfort (ID 759), maintenance of faecal nitrogen content and/or normal blood urea concentrations (ID 840, 1975), and maintenance of normal blood LDL-cholesterol concentrations (ID 841) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). EFSA Journal 9(4), 2022.Available at: http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2011.2022/epdf
- EFSA (2014). Scientific Panel on Food Additives and Nutrient Sources added to Food (ANS). Minutes of the 49th plenary meeting. Held on 4-6 February 2014, Parma, Italy. (Agreed on 3 April 2014). EFSA/FIP/277. Available at http://www.efsa.europa.eu/sites/default/files/event/140204-m.pdf
- EFSA (2017). European Food Safety Authority. Re-evaluation of acacia gum (E 414) as a food additive. EFSA J. 15(4), 4741. Available at http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2017.4741/epdf
- EFSA (2019). European Food Safety Authority. Opinion on the re-evaluation of acacia gum (E 414) as a food additive in foods for infants below 16 weeks of age and the follow-up of its re-evaluation as a food additive for uses in foods for all population groups. EFSA Panel on Food Additives and Flavourings (FAF). Question Number: EFSA-Q-2018-00525. EFSA

Journal 17(12), 5922. DOI: 10.2903/j.efsa.2019.5922. Available at https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2019.5922

- Elbashir SMI et al. (2018). Free radical scavenging,α-glucosidase inhibitory and lipase inhibitory activities of eighteen Sudanese medicinal plants. BMC Complement. Altern. Med. 18(1), 282. DOI: 10.1186/s12906-018-2346-y. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30340582
- El-Garawani I et al.(2021).The Ameliorative Role of Acacia senegal Gum against the Oxidative Stress and Genotoxicity Induced by the Radiographic Contrast Medium (Ioxitalamate) in Albino Rats. Antioxidants (Basel) 10(2), 221. DOI: 10.3390/antiox10020221. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33540787/
- European Commission (undated). Food additives database. Available at:https://ec.europa.eu/food/food-feed-portal/screen/food-additives/search
- FAO/WHO Food standards (2019). Codex Alimentarius. The 42ndSession of the Codex Alimentarius Commission. Record for gum arabic. Available at https://www.fao.org/gsfaonline/additives/details.html?id=63
- FDA (2024a). US Food and Drug Administration. Substances Added to Food (formerly EAFUS). Last updated 29 October 2024. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/?set=FoodSubstances
- FDA (2024b). US Food and Drug Administration. Inactive Ingredient Database. Last updated 19 October 2024. Available at https://www.accessdata.fda.gov/scripts/cder/iig/index.cfm
- GENE-TOX (1998). Record for gum Arabic. GENETOX record no. 2904. Last revision date 23 April 1998 (records no longer being updated after 1998).Available at https://www.toxinfo.io/
- GESTIS (undated). GESTIS Substance Database. Record for gum arabic (CAS RN 9000-01-5). Available at https://www.dguv.de/ifa/gestis/gestis-stoffdatenbank/index-2.jsp
- Glover D. A. et al.(2009) “Acacia (sen) SUPERGUM™ (Gum arabic): An evaluation of potential health benefits in human subjects” Food Hydrocolloids, 23(8): 2410–2415.Available at: http://www.sciencedirect.com/science/article/pii/S0268005X09001416
- Gouda, E., & Babiker, F. (2022). Gum Arabic protects the rat heart from ischemia/reperfusion injury through anti-inflammatory and antioxidant pathways.Scientific reports,12(1), 17235. https://doi.org/10.1038/s41598-022-22097-0
- Hall RL and Oser BL (1965). Recent progress in the consideration of flavoring ingredients under the food additives amendment. III. GRAS substances. Food Technology 19(2), 151-197. Available at https://www.femaflavor.org/sites/default/files/3. GRAS Substances%282001-3124%29_0.pdf
- Hammad FT et al. (2019). The effect of arabic gum on renal function in reversible unilateral ureteric obstruction. Biomolecules 9(1), E25. DOI: 10.3390/biom9010025. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30641998
- Health Canada (2021). Drugs and Health Products. Natural Health Products Ingredients Database. Records for acacia, acacia senegal gum and acacia arabica (no CAS RNs). Last updated 7 December 2021. Available at http://webprod.hc-sc.gc.ca/nhpid-bdipsn/search-rechercheReq.do?lang=eng
- Hernández-García E et al.(2019). Chemical composition of Acacia farnesiana (L) wild fruits and its activity against Mycobacterium tuberculosis and dysentery bacteria. J. Ethnopharmacol. 230, 74-80. DOI: 10.1016/j.jep.2018.10.031. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30367988
- Hilmi Y et al. (2014). A study of antioxidant activity, enzymatic inhibition and in vitro toxicity of selected traditional Sudanese plants with anti-diabetic potential. BMC Complement. Altern. Med. 14, 149. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/24885334

- Hoshino T et al. (2019). Impact of Acacia bark extract tablets on the skin of healthy humans: a randomized, double-blind, placebo-controlled study. Biosci. Biotechnol. Biochem. 83(3), 538-550. DOI: 10.1080/09168451.2018.1547626. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30526388
- HSDB (2002). Record for acacia. Hazardous Substances Databank Number: 1914. Last Revision Date: 13 May 2002. Available at https://www.toxinfo.io/
- Hu JL et al. (2014).Effect of gum arabic on glucose levels and microbial short-chain fatty acid production in white rice porridge model and mixed grain porridge model.J. Agric. Food Chem. 62(27), 6408-16. PubMed, 2015 available at http://www.ncbi.nlm.nih.gov/pubmed/24941348
- Ibrahim RM et al. (2020). Arabic gum ameliorates histological and histochemical changes in the renal cortex of rats induced by alloxan injection. Journal of Medical and Life Science 2(4), 69-90. DOI: 10.21608/jmals.2020.133543. Available at https://journals.ekb.eg/article_133543.html
- IFRA (undated). International Fragrance Association. IFRA Transparency List. Available at: https://ifrafragrance.org/
- Jaafar NS (2019). Clinical effects of Arabic gum (Acacia): A mini review.Iraqi J. Pharm. Sci. 28(2), 9-16. DOI: https://doi.org/10.31351/vol28iss2pp9-16. Available at http://bijps.uobaghdad.edu.iq/index.php/bijps/article/view/896
- Jadhav SB et al. (2014).Enhanced stability of alcohol dehydrogenase by non-covalent interaction with polysaccharides.Appl. Microbiol. Biotechnol. 98(14), 6307-16. PubMed, 2015 available at http://www.ncbi.nlm.nih.gov/pubmed/24658590
- JECFA (1982). Twenty-sixth Report of the Joint FAO/WHO Expert Committee on Food Additives. Toxicological evaluation of certain food additives. Arabic gum. WHO Food Add. Ser. 17. Available at http://www.inchem.org/documents/jecfa/jecmono/v17je06.htm
- JECFA (2010). Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Record for gum Arabic. Available at http://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID=865
- JECFA (2019). Safety evaluation of certain food additives.WHO Food Additives Series 75. Prepared by the eighty-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives. FAO JECFA Monographs. Available at https://apps.who.int/iris/bitstream/handle/10665/312367/9789241660754-eng.pdf?ua=1
- JTI Heated Tobacco Stick Study Report(s)
- JTI NTM Study Report
- Kaddam L et al. (2017). Gum Arabic as novel anti-oxidant agent in sickle cell anemia, phase II trial. BMC Hematol. 17, 4. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28331623
- Kaddam LAG et al. (2019). Biochemical effects and safety of Gum arabic (Acacia Senegal) supplementation in patients with sickle cell anemia. Blood Res. 54(1), 31-37. DOI: 10.5045/br.2019.54.1.31. Available at https://synapse.koreamed.org/search.php?where=aview&id=10.5045/br.2019.54.1.31&code=3072BR&vmode=FULL
- Kandeal HAM et al. (2021). The Possible Radio Protective Role of Gum Arabic on The Kidney Cortex of Adult Male Albino Rats. The Egyptian Journal of Hospital Medicine 82(2), 256-269. DOI: 10.12816/EJHM.2021.143886. Available at https://ejhm.journals.ekb.eg/article_143886.html
- LabstatInternational Inc. (2023a). Characterization of Heat-not-Burn Emissions.Analytical Test Report(s).
- LabstatInternational Inc. (2023b). 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).

- Lakshmi T et al. (2018). Seed and bark extracts of Acacia catechu protects liver from acetaminophen induced hepatotoxicity by modulating oxidative stress, antioxidant enzymes and liver function enzymes in Wistar rat model. Biomed. Pharmacother. 108, 838-844. DOI: 10.1016/j.biopha.2018.08.077. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30372895
- Larson R et al. (2021). Acacia Gum Is Well Tolerated While Increasing Satiety and Lowering Peak Blood Glucose Response in Healthy Human Subjects. Nutrients 13(2), 618. DOI: 10.3390/nu13020618. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33672963/
- Magnini RD et al. (2021BMC Complement Med Ther2021.Toxicity and bacterial anti-motility activities of the hydroethanolic extract of Acacia senegal (L.) Willd (Fabaceae) leavesAvailable at https://pubmed.ncbi.nlm.nih.gov/34187452/
- Mambe FT et al. (2019). Antibacterial and antibiotic modifying potential of crude extracts, fractions, and compounds from Acacia polyacantha Willd. against MDR gram-negative bacteria. Evidence-Based Complementary and Alternative Medicine. Article ID 7507549. DOI: 10.1155/2019/7507549. Available at https://www.hindawi.com/journals/ecam/2019/7507549/cta/
- Martindale (1999). The Extra Pharmacopoeia. Edited by K Parfitt.Thiry-second edition.The Pharmaceutical Press. ISBN 0-85369-429-X.
- McLean Ross AH et al. (1984). A study of the effects of dietary gum arabic in the rat.Br J Nutr. 1984 Jan;51(1):47-56.(PubMed 6317010)
- Merck (2013). The Merck Index. An encyclopaedia of chemicals, drugs and biologicals. Fifteenth edition. O'Neil MJ et al. ed. Royal Society of Chemistry, Cambridge, UK
- Mohammed ME et al. (2020). Effect of Acacia senegal on TGF-β1 and vascular mediators in a rat model of diabetic nephropathy. Arch. Physiol. Biochem. Epub ahead of print. DOI: 10.1080/13813455.2020.1781901. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32574082/
- Nasir O (2013).Renal and extrarenal effects of Gum Arabic (Acacia Senegal)--what can be learned from animal experiments?Kidney Blood Press. Res. 37(4-5), 269-79. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24022265
- Nasir O et al. (2008). Effects of gum arabic (Acacia senegal) on water and electrolyte balance in healthy mice.J Ren Nutr. 2008 Mar;18(2):230-8.(PubMed 18267216)
- Nasir O et al. (2020). Comparative efficacy of Gum Arabic (Acacia senegal) and Tribulus terrestris on male fertility. Saudi Pharm. J. 28(12), 1791-1796. DOI: 10.1016/j.jsps.2020.11.005. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33424268/
- Nemmar A et al. (2019). Gum arabic ameliorates impaired coagulation and cardiotoxicity induced by water-pipe smoke exposure in mice. Front. Physiol. 10, 53. DOI: 10.3389/fphys.2019.00053. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30858803
- NTP (2022) National Toxicology Program.Testing Status of Gum Arabic 10751-P. CASRN 9000-01-5. Upataded 4 January 2022. Available at https://ntp.niehs.nih.gov/whatwestudy/testpgm/status/ts-10751-p.html?utm_source=direct&utm_medium=prod&utm_campaign=ntpgolinks&utm_term=ts-10751-p
- OECD. Organisation for Economic Cooperation and Development. The Global Portal to Information on Chemical Substances (eChemPortal). Gum arabic (CAS RN 9000-01-5). Available at: http://webnet.oecd.org/CCRWeb/Search.aspx
- Osman HAE-FM and Zaki MS (2019). Antibacterial activity of Arabic Gum against some pathogenic and non pathogenic bacteria (Review). Researcher 11(2), 17-23. DOI: 10.7537/marsrsj110219.05. Available at http://www.sciencepub.net/researcher/rsj110219/05_34449rsj110219_17_23.pdf

- Pai MK et al. (2013).Gum Arabic enhances paracellular transport of water in amphibian everted small intestinal segments.Indian J. Physiol. Pharmacol. 57(2), 195-8. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24617171
- Patyra, Andrzej et al. (2024) “Pharmacological and phytochemical insights on the pancreatic β-cell modulation by Angelica L. roots.” Journal of ethnopharmacology vol. 329 (2024): 118133. doi:10.1016/j.jep.2024.118133
- Philips A. O. et al., (2011) “Biofunctional behaviour and health benefits of a specific gum arabic” Food Hydrocolloids; 25(2):165–169.Available at: http://www.sciencedirect.com/science/article/pii/S0268005X10000585
- PubChem (2023). Record for CAS 9000-01-5. Created 17 March 2015. Last modified 28 January 2023. Available at https://pubchem.ncbi.nlm.nih.gov/compound/91333377
- Rehman KU et al. (2003). Gum arabic (GA) modifies paracellular water and electrolyte transport in the small intestine.Dig Dis Sci. 2003 Apr;48(4):755-60.(PubMed 12741467)
- RTECS (2013). Registry of Toxic Effects of Chemical Substances. Record for arabic gum (CAS RN 9000-01-5).Last updated July 2013.
- Slavin J et al. (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
- Taha MS et al. (2020). Anti-ulcerogenic activity of Gum Arabic in gastric mucosal injury induced by ethanol in male albino rats. Appl. Physiol. Nutr. Metab. 45(7), 731-736. DOI: 10.1139/apnm-2018-0233. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/31905015/
- Tschannen MP et al. (2017). Thaumatin and gum arabic allergy in chewing gum factory workers. Am. J. Ind. Med. 60(7), 664-669. DOI: 10.1002/ajim.22729. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28543634
- US EPA (2024). Safer Chemical Ingredients List. Last updated 30 September 2024. Available at https://www.epa.gov/saferchoice/safer-ingredients
- US EPA ECOTOX Database. Record for gum arabic.
- US EPA InertFinder Database (2024). Last updated 29 February 2024. Available athttps://iaspub.epa.gov/apex/pesticides/f?p=INERTFINDER:1:0::NO:1
- US EPA Substance Registry Services (SRS) – TSCA and CDR lists. Available at https://cdxapps.epa.gov/oms-substance-registry-services/search
- Viinanen et al. (2011). Gum Arabic as a Cause of Occupational Allergy.J Allergy (Cairo).2011; 2011: 841508. (PubMed PMC3124965)
- Xuan NT et al. (2010). Stimulation of mouse dendritic cells by Gum Arabic.Cell Physiol Biochem.2010;25(6):641-8 (PubMed 20511709).
- Zingue S et al. (2018). The hydro-ethanolic extract of Acacia seyal (Mimosaceae) stem barks induced death in an ER-negative breast cancer cell line by the intrinsic pathway of apoptosis and inhibited cell migration. J. Ethnopharmacol. 223, 41-50. DOI: 10.1016/j.jep.2018.05.021. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29783017

12. Other information

- (1981). Gum acacia. Ch. Joubert. Int. Off. of Cocoa and Choc., Int. Sug. Conf. Manuf. Assn, General Assembly, Official Report, Hershey, PA, USA, 12 May 1981, 44-46.
- Adiotomre J et al. (1990). Dietary fiber: in vitro methods that anticipate nutrition and metabolic activity in humans.American Journal of Clinical Nutrition 52, 128.
- Ali BH et al. (2009). Biological effects of gum arabic: a review of some recent research.Food and Chemical Toxicology 47, 1.

- Ali BH et al. (2013). Comparative efficacy of three brands of gum acacia on adenine-induced chronic renal failure in rats. Physiol. Res. 62(1), 47-56. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23173676
- Ali BH et al. (2013). Effect of gum arabic on oxidative stress and inflammation in adenine-induced chronic renal failure in rats. PloS One 8(2), e55242. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23383316
- Ali BH et al. (2013). New model for adenine-induced chronic renal failure in mice, and the effect of gum acacia treatment thereon: comparison with rats. J. Pharmacol. Toxicol. Methods 68(3), 384-93. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23669035
- Ali BH et al. (2014). Anemia in adenine-induced chronic renal failure and the influence of treatment with gum acacia thereon. Physiol. Res. 63(3), 351-8. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24564605
- Al-Othman AA et al.(1999). Plasma total, lipoprotein cholesterol, organs cholesterol and growth performance in rats fed dietary gum arabic.Fd Chemy 62, 69.
- Anderson DMW (1986). Evidence for the safety of gum arabic (Acacia senegal (L.) Willd.) as a food additive - a brief review. Food Additives and Contaminants 3, 225.
- Anderson DMW (1988). The natural gums.BNF Nutrition Bulletin 13, 101.
- Anderson DMW (1991). Food safety assurance: revised specification for gum arabic.British Food Journal 93,2.
- Anderson DMW et al. (1982). Subchronic effects of gum arabic (Acacia) in the rat.Toxicology Letters 14,221.
- Anderson DMW et al. (1984). Transmission electron microscopy of heart and liver tissues from rats fed with gums arabic and tragacanth.Toxicology Letters 21, 83.
- Anderson DMW et al. (1985). The amino acid composition of the proteinaceous component of gum arabic (Acacia senegal (L.)Willd.).Food Additives and Contaminants 2, 159.
- Anderson DMW et al. (1986). Transmission electron microscopy of jejunum, ileum, and caecum tissues from rats fed with gums arabic, karaya and tragacanth.Toxicology 41, 75.
- Anderson DMW et al. (1990). Specifications for gum arabic (Acacia senegal); analytical data for samples collected between 1904 and 1989.Food Additives and Contaminants 7, 303.
- Anderson DMW et al. (1991). Gum arabic (Acacia senegal): unambiguous identification by 13C-NMR spectroscopy as an adjunct to the revised JECFA specification, and the application of 13C-NMR spectra for regulatory/legislative purposes.Food Additives and Contaminants 8, 405.
- Anderson DMW McDougall FJ (1987). Degradative studies of gum arabic (Acacia senegal (L.) Willd.) with special reference to the fate of the amino acids present.Food Additives and Contaminants 4, 247.
- Anderson DMW McDougall FJ (1987). Degradative studies of highly proteinaceous Acacia gum exudates.Food Additives and Contaminants 4, 257.
- Anderson DMW Morrison NA (1990). Identification of Albizia gum exudates which are not permitted food additives.Food Additives and Contaminants 7, 175.
- Anderson DMW Morrison NA (1990). The identification of Combretum gums which are not permitted food additives, II.Food Additives and Contaminants 7, 181.
- Anderson DMW Yin XS (1988). The amino acid composition and quantitative sugar-amino acid relationships in sequential Smith-degradation products from gum talha (Acacia seyal Del.)Food Additives and Contaminants 5, 1.
- Annison G et al. (1995). Feeding Australian acacia gums and gum arabic leads to non-starch polysaccharide accumulation in the cecum of rats.Journal of Nutrition 125, 283.
- Anon (1981). Eleven carcinogenicity reports approved.Food Chemical News 23, 6.
- Anon (1982). Final NTP bioassay results.Food Chemical News 24, 23
- Anon (1988). Data on substances to be reviewed by FAO/WHO requested.Food Chemical News 30, 22 26.

- Anon (1996). Proposal weakens additive standard.Food Magazine 32, 4.
- Babiker R et al. (2012). Effects of gum arabic ingestion on body mass index and body fat percentage in healthy adult females: two-arm randomized, placebo controlled, double-blind trial.Nutrition Journal (available at:). http://www.nutritionj.com/content/11/1/111
- Bachmann E et al. (1978). Biochemical effects of gum arabic, gum tragacanth, methylcellulose and carboxymethylcellulose-Na in rat heart and liver.Pharmacology 17, 39.(10.1159/000136833).
- Bachmann E Zbinden G (1978). Biochemical effects of chronically administered suspending agents on mitochondrial metabolism and hepatic mixed function oxidases in rat, mice, and hamster.Archives of Toxicology 1,183.
- Bailey DE et al. (1976). Comparative acute oral toxicity of flavor materials when dosed singly or in combination to the rat.Toxicology and Applied Pharmacology 37, 143.
- Bliss DZ et al. (1996). Supplementation with gum arabic fiber increases fecal nitrogen excretion and lowers serum urea nitrogen concentration in chronic renal failure patients consuming a low-protein diet.American Journal of Clinical Nutrition 63, 392.
- BNF RSC (1997). Functional foods 97: The consumer the products and the evidence.Abstracts presented at Conference on 2-4 April 1997, Wye College, Kent.
- Bohner CB et al. (1941). Sensitivity of gum acacia, with a report of ten cases of asthma in printers.Journal of Allergy 12, 290.
- Bohner CB et al. (1941). Sensitivity to gum acacia, with a report of ten cases of asthma in printers.Journal of Allergy 12, 290.
- Booth AN et al. (1963). Physiologic effects of three microbial polysaccharides on rats.Toxicology and Applied Pharmacology 5, 478.
- Bourquin LD et al. (1996). Fermentation of various dietary fiber sources by human fecal bacteria.Nutrition Research 16, 1119.
- Bueno AR et al. (2000). Feline colonic morphology and mucosal tissue energetics as influenced via the source of dietary fiber.Nutrition Research 20, 985.
- Burry JN et al. (1969). The value of patch testing: a review of 363 cases of allergic contact dermatitis.Medical Journal of Australia 1, 1226.
- Calame W et al. (2008). Gum arabic establishes prebiotic functionality in healthy human volunteers in a dose-dependent manner.British Journal of Nutrition 100, 1269.(10.1017/S0007114508981447).
- Campbell JM et al. (1997). Metabolic characteristics of healthy adult males as affected by ingestion of a liquid nutritional formula containing fish oil, oligosaccharides, gum arabic and antioxidant vitamins.Food and Chemical Toxicology 35, 1165.
- Canadian Thoracic Society (1989). Occupational asthma: recommendations for diagnosis, management and assessment of impairment.Canadian Medical Association Journal 140, 1029.
- Carlson AJ Hoelzel F (1948). Prolongation of the life span of rats by bulk-formers in the diet.Journal of Nutrition 36,27.
- Chiang DT et al. (2002). Prevalence of arabic and karaya gum allergy in latex-allergic patients.Journal of Allergy and Clinical Immunology 109, S333.
- Chinachoti P (1995). Carbohydrates: functionality in foods. American Journal of Clinical Nutrition 61, 922S.
- CIR (2005). Final report of the safety assessment of Acacia catechu gum, Acacia concinna fruit extract, Acacia dealbata leaf extract, Acacia dealbata leaf wax, Acacia decurrens extracts, 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 24, Suppl.3 75.
- Codipilly CN et al. (2006). Enhancement of absorption by gum arabic in a model of gastrointestinal dysfunction.Journal of the American College of Nutrition 25, 307.

- Collins TFX et al. (1987). Study of the teratogenic potential of gum arabic.Food and Chemical Toxicology 25,815.
- Commission (2005). Provisional list of monomers and additives notified to European Commission as substances which may be used in the manufacture of plastics or coatings intended to come into contact with foodstuffs.Synoptic Document (updated to June 2005). Additives (pages 114-297).
- Correia O et al. (1992). Airborne contact dermatitis from the woods Acacia melanoxylon and Entandophragma cylindricum.Contact Dermatitis 27, 343.
- Cun C et al. (1991). A contribution to the study on the relationship between certain polysaccharides and calcium ions.Annales des Falsifications et de l'Expertise Chimique 84, 261.
- Davidson MH et al. (1998). A low-viscosity soluble-fiber fruit juice supplement fails to lower cholesterol in hypercholesterolemic men and women.Journal of Nutrition 128, 1927.
- de las Heras M et al.(1999). Outcome of occupational asthma due to arabic gum.Journal of Allergy and Clinical Immunology 103, S221.
- Deutsche Forschungsgemeinschaft (1999). Occupational toxicants. Occupational Toxicants. Critical Data Evaluation for MAK Values and Classification of Carcinogens.Volume 13.VCH Publishers.
- Doi Y et al. (2006). A ninety-day oral toxicity study of a new type of processed gum arabic, from acacia tree (Acacia senegal) exudates, in F344 rats.Food and Chemical Toxicology 44, 560.
- Eastwood M (1987). Dietary fibre and serum lipids.Scand. J. Gastroent.22, Suppl.129 210.
- Eastwood MA (1992). The physiological effect of dietary fiber: An update.Annual Review of Nutrition 12,19.
- Eastwood MA et al. (1986). The effect of the polysaccharide composition and structure of dietary fibers on cecal fermentation and fecal excretion.American Journal of Clinical Nutrition 44, 51.
- EFSA (2004). Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission related to the use of certain food additives in jelly mini cups (Question number EFSA-Q-2004-054).(Adopted on 12 July 2004).EFSA Journal 82, 1.
- EFSA (2010). EFSA Panel on Food Additives and Nutrient Sources added to Food; Scientific Opinion on the use of gum acacia modified with octenyl succinic anhydride (OSA) as a food additive. EFSA Journal 8, 3 1539. (available at:). http://www.efsa.europa.eu/en/scdocs/doc/1539.pdf
- EFSA (2011). Consolidated list of Article 13 health claims. List of references received by EFSA. Part 1. IDs 1-1000. 5 April 2011 Internet (available at: https://www.efsa.europa.eu/sites/default/files/topic/ndaart13ref01.pdf
- EFSA (2011). Consolidated list of Article 13 health claims. List of references received by EFSA. Part 2. IDs 1001-2000. 5 April 2011 Internet (available at: https://www.efsa.europa.eu/sites/default/files/topic/ndaart13ref02.pdf
- EFSA (2017). European Food Safety Authority. Re-evaluation of acacia gum (E 414) as a food additive. EFSA J. 15(4), 4741. Available at http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2017.4741/epdf
- El-Mawla AMAA Osman HEH (2011). Effects of gum acacia aqueous extract on the histology of the intestine and enzymes of both the intestine and the pancreas of albino rats treated with Meloxicam.Pharmacognosy Research 3, 114.(10.4103/0974-8490.81959).
- Elsenhans B et al. (1981). Long-term feeding of unavailable carbohydrate gelling agents. Influence of dietary concentration and microbiological degradation on adaptive responses in the rat.American Journal of Clinical Nutrition 34, 1837.

- Fahey GC Campbell JM (1998). Metabolic characteristics of healthy adult males as affected by ingestion of a liquid nutritional formula containing fish oil, oligosaccharides, gum arabic and antioxidant vitamins.Food and Chemical Toxicology 36, 1032.
- FAO (1966). Acacia Gum Identification Tests (DRAFT DOCUMENT) Prepared for Tenth Session of FAO/WHO Expert Committee on Food Additives, Geneva 11-18 October 1966, 2nd Draft, p.1.
- FAO/WHO (1959). Acacia gum. Industrial Gums, Polysaccharides and their Derivatives, Whistler R.L. and Bemiller J.N. (eds), Academic Press, New York and London, pp.279.
- FAO/WHO (1970). Arabic gum. 13th Report of the Joint FAO/WHO Expert Committee on Food Additives, FAO Nutrition Meetings Report Series No.46A, WHO/FOOD ADD/70.36, p.97.
- FAO/WHO (1982). Evaluation of certain food additives and contaminants. Twenty-sixth Report of the Joint FAO/WHO Expert Committee on Food Additives, World Health Organisation Technical Report Series 683, Geneva, p.27-29.
- FAO/WHO (1982). Toxicological evaluation of certain food additives. International Programme on Chemical Safety, IPCS. Twenty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives, Geneva, WHO Tech.Rep. Ser. No.683, 1982.
- FASEB (1973). Evaluation of the health aspects of gum arabic as a food ingredient.NTIS PB234904, FDABFGRAS207.
- FDA (1974). Gum arabic (acacia). Federal Register 39, 34203.
- FDA (2005). Food additives permitted for direct addition to food for human consumption; acacia (gum arabic).21 CFR Part 172 Federal Register 70, 8032.
- FDA (2008). Agency Response Letter: GRAS Notice No.GRN 000247.
- FDA (2010). United States Pharmacopeial Convention; filing of food additive petition. FDA-2010-F-0320 Federal Register 75, 48353. (available at:). http://edocket.access.gpo.gov/2010/pdf/2010-19722.pdf
- FDA (2011). Nexira; filing of food additive petition. FDA-2011-F-0765 Federal Register 76, 78866. (available at:http://www.gpo.gov/fdsys/pkg/FR-2011-12-20/pdf/2011-32542.pdf).
- Feinberg SM Schoenkerman BB (1940). Karaya and related gums as causes of atopy.Wisconsin Medical Journal 39,734.
- Ferguson LR et al. (1995). The effects of a soluble-fibre polysaccharide on the adsorption of carcinogens to insoluble dietary fibres.Chemico-Biological Interactions 95, 245.
- Fiorini G et al. (1990). Symptoms of respiratory allergies are worse in subjects with coexisting food sensitization.Clinical and Experimental Allergy 20, 689.
- Flickinger EA et al. (2000). Glucose-based oligosaccharides exhibit different in vitro fermentation patterns and affect in vivo apparent nutrient digestibility and microbial populations in dogs.Journal of Nutrition 130,1267.
- Fotisch K et al. (1998). IgE antibodies specific for carbohydrates in a patient allergic to gum arabic (Acacia senegal).Allergy 53,1043.
- Fournier PE et al. (1987). In vivo determination of the caloric value of gum arabic in the male rat-biological data.Food Additives and Contaminants 4, 233.
- Fowler PBS (1952). Printers' asthma. Lancet ii 755.
- Fowler PBS (1952). Printers' asthma. Lancet ii, 755.
- Frohberg H et al. (1969). On the mechanism of the fetotoxic effect of tragacanth.Archiv f-++r Toxikologie 25,268.
- Gad SC et al. (2006). Nonclinical vehicle use in studies by multiple routes in multiple species.International Journal of Toxicology 25, 499.
- Gado AM & Aldahmash BA (2013). Antioxidant effect of Arabic gum against mercuric chloride-induced nephrotoxicity. Drug Des. Devel. Ther. 7, 1245-52. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24174869
- Gelfand HH (1943). The allergenic properties of the vegetable gums. A case of asthma due to tragacanth. Journal of Allergy 14, 203.

- Gelfand HH (1949). The vegetable gums by ingestion in the etiology of allergic disorders.Journal of Allergy 20,311.
- Gohl B Gohl I (1977). The effect of viscous substances on the transit time of barley digesta in rats.Journal of the Science of Food and Agriculture 28, 911.
- Green S (1977). Present and future uses of mutagenicity tests for assessment of the safety of food additives.Journal of Environmental Pathology and Toxicology 1, 49.
- Hall RL Oser BL (1965). Recent progress in the consideration of flavoring ingredients under the Food Additives Amendment.III. GRAS substance.Food Technology 19, 151.
- Hall WK et al. (1940). Studies on the intravenous injection of colloids II. Effects of gum acacia on certain functions of the liver with a note on its effects on the production of immume bodies.Journal of Laboratory and Clinical Medicine 26, 330.
- Hall WK et al. (1940). Studies on the intravenous injection of colloids. II. Effects of gum acacia on certain functions of the liver with a note on its effects on the production of immune bodies.Journal of Laboratory and Clinical Medicine 26, 330.
- Hallman JE et al. (1996). Colonic mucosal tissue energetics and electrolyte transport in dogs fed cellulose, beet pulp or pectin/gum arabic as their primary fiber source.Nutrition Research 16, 303.
- Harington JS (1963). Some biological actions of silica: their part in the pathogenesis of silicosis.South African Medical Journal 37, 451.
- Harris PJ et al. (1993). The effects of soluble-fiber polysaccharides on the adsorption of a hydrophobic carcinogen to an insoluble dietary fiber.Nutrition and Cancer 19, 43.
- Haskell WL et al. (1992). Role of water-soluble dietary fiber in the management of elevated plasma cholesterol in healthy subjects.American Journal of Cardiology 69, 433.
- Hefle SL et al. (1996). Allergenic foods. CRC Critical Reviews in Food Science and Nutrition 36, S69.
- Hosobuchi C et al. (1999). Efficacy of acacia, pectin, and guar gum-based fiber supplementation in the control of hypercholesterolemia.Nutrition Research 19, 643.
- Howard MD et al. (1995). Dietary fructooligosaccharide, xylooligosaccharide and gum arabic have variable effects on cecal and colonic microbiota and epithelial cell proliferation in mice and rats.Journal of Nutrition 125, 2604.
- Hurley HJ Shelley WB (1959). The colloidal state as a stimulus for non-allergic epithelioid granulomas: experimental studies in man with pure sodium stearate and palmitate.Journal of Investigative Dermatology 33, 203.
- Ilchyshyn A Smith AG (1985). Gum arabic sensitivity associated with epidemic hysteria dermatologica.Contact Dermatitis 13,282.
- Ink SL Hurt HD (1987). Nutritional implications of gums.Food Technology 41, 77.
- Ishizaki M Ueno S (1987). The DNA-damaging activity of natural food additives (IV).Journal of the Food Hygienics Society of Japan 28, 498.
- Jaafar NS (2019). Clinical effects of Arabic gum (Acacia): A mini review. Iraqi J. Pharm. Sci. 28(2). Available at. http://bijps.uobaghdad.edu.iq/index.php/bijps/article/view/896
- JECFA (1970). Specifications for the identity and purity of some food colours, emulsifiers, stabilizers, anti-caking agents and certain other food additives. Thirteenth Report of the Joint FAO/WHO Expert Committee Report on Food Additives.F.A.O. Nutrition Meetings Report Series 46B
- JECFA (1970). Toxicological evaluation of some food colours, emulsifiers, stabilizers, anti-caking agents and certain other substances. Thirteenth Report of the Joint FAO/WHO Expert Committee on Food Additives.F.A.O. Nutrition Meetings Report Series 46A
- JECFA (1974). Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers and thickening agents. Seventeenth Report of the Joint FAO/WHO Expert Committee on Food Additives.WHO Food Additive Series 5
- JECFA (1982). Evaluation of certain food additives and contaminants. Twenty-sixth Report of the Joint FAO/WHO Expert Committee on Food Additives.683

- JECFA (1982). Toxicological evaluation of certain food additives. Twenty-sixth Report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Food Additive Series 17 (available at:). http://www.inchem.org/documents/jecfa/jecmono/v17je25.htm
- JECFA (1989). Temporary ADIs for some additives dropped, more data sought.Food Chemical News 31,31 43.
- JECFA (1990). Evaluation of certain food additives and contaminants. Thirty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives.WHO Technical Report 789
- JECFA (1990). Specifications for identity and purity of certain food additives. Emulsifiers, enzyme preparations, flavouring agents, food colours, thickening agents, miscellaneous food additives.FAO Food and Nutrition Paper 49
- JECFA (1990). Toxicological evaluation of certain food additives and contaminants. Thirty-fifth meeting of the joint FAO/WHO Expert Committee on Food Additives.WHO Food Additive Series 26
- JECFA (1992). Compendium of food additive specifications, Volume 1. Combined specifications from the first to the thirty-seventh meeting 1956-1990.FAO Food and Nutrition Paper 52
- JECFA (1995). Compendium of food additive specifications. Addendum 3. Joint FAO/WHO Expert Committee on Food Additives. 44th session, Rome, 14-23 February 1995. FAO Food and Nutrition Paper 52 Add.3
- JECFA (2010). Evaluation of certain food additives. Joint FAO/WHO Expert Committee on Food Additives. Seventy-first meeting. Geneva, 16 to 24 June 2009 WHO Technical Report 956 (available at:). http://whqlibdoc.who.int/trs/WHO_TRS_956_eng.pdf
- JECFA (2010). Safety evaluation of certain food additives. Joint FAO/WHO Expert Committee on Food Additives. Seventy-first meeting. Geneva, 16 to 24 June 2009. WHO Food Additive Series 62 (available at:). http://whqlibdoc.who.int/publications/2010/9789241660624_eng.pdf
- JECFA (2011). Evaluation of certain food additives and contaminants. Seventy-fourth report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, 14-23 June 2011 WHO Technical Report 966 (available at:). http://whqlibdoc.who.int/trs/WHO_TRS_966_eng.pdf
- JECFA (2012). Safety evaluation of certain food additives and contaminants. Seventy-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives. Rome, 14-23 June 2011 WHO Food Additive Series 65 (available at:). http://whqlibdoc.who.int/publications/2012/9789241660655_eng.pdf
- JECFA (2012). Seventy-seventh JECFA meeting: Food Additives and Contaminants. List of substances scheduled for evaluation and request for data. Seventy-seventh meeting of the Joint FAO/WHO Expert Committee on Food Additives. Rome, 4-13 June 2013. Published 21 September 2012 Internet (available at:). http://www.fao.org/fileadmin/user_upload/agns/pdf/jecfa/JECFA77_call_data.pdf
- Jesen CD et al. (1993). The effect of acacia gum and a water-soluble dietary fiber mixture on blood lipids in humans.Journal of the American College of Nutrition 12, 147.
- Johnson IT Gee JM (1991). Influence of soluble non-starch polysaccharides and food intake on mucosal cell turnover and some gastrointestinal peptides in the rat.Proceedings of the Nutrition Society 50, 57A.
- Kawase A et al. (2007). Gum arabic enhances intestinal calcium absorption in rats.Journal of Health Science 53, 622.
- Koseki M et al. (1989). Effects of gum arabic and pectin on the emulsification, the lipase reaction, and the plasma cholesterol level in rats.Agricultural and Biological Chemistry 53, 3127.
- Kravtchenko TP (1998). Acacia gum - a natural soluble dietary fibre. Functional Foods. The Consumer, the Products and the Evidence. Royal Society of Chemistry.
- Kritchevsky D (1987). Dietary fibre and lipid metabolism.International Journal of Obesity 11, 33.

- Kudoh K et al. (1998). Effect of indigestible saccharides on B lymphocyte response of intestinal mucosa and cecal fermentation in rats.Journal of Nutritional Science and Vitaminology 44, 103.
- Lomax AR Calder PC (2009). Prebiotics, immune function, infection and inflammation: a review of the evidence.British Journal of Nutrition 101, 633
- MAFF (1968). Report on further classes of food additives.FAC Report 7
- MAFF (1970). Food Additives and Contaminants Committee report on the review of the Emulsifiers and Stabilisers in Food Regulations 1962.FAC Report 9
- MAFF (1976). Food Additives and Contaminants Committee report on the review of flavourings in food.Appendix 2 FAC Report 22,
- MAFF (1978). Food Additives and Contaminants Committee Report on the review of additives and processing aids used in the production of beer.FAC Report 26
- MAFF (1992). Food Advisory Committee report on the review of the Emulsifiers and Stabilisers in Food Regulations.FAC Report FdAC/REP/11
- Malkki Y Cummings JH (1996). COST Action 92: Dietary fibre and fermentation in the colon. Proceedings of COST Action 92 workshop, Espoo, Finland, 15 to 17 June 1995, Luxembourg, pp.424.
- Mallett AK et al. (1984). Hydrocolloid food additives and rat caecal microbial enzyme activities.Food and Chemical Toxicology 22, 415.
- Maxwell WA Newell GW (1974). Screening techniques for environmental mutagens. Molecular and Environmental Aspects of Mutagenesis. Ed. L. Prakash et al.C.C. Thomas, Springfield.
- Maxwell WA Newell GW (1975). Screening techniques for environmental mutagens. Molecular and Environmental Aspects of Mutagenesis. Prakash L., et al.(Eds)
- Maytum CK Magath TB (1932). Sensitivity to acacia. Journal of the American Medical Association 99, 2251.
- Maytum CK Magath TB (1932). Sensitivity to acacia. Proceedings of the Staff Meetings of the Mayo Clinic 7, 216.
- Maytum CK Magath TB (1932). Sensitivity to acacia. Proceedings of the Staff Meetings of the Mayo Clinic 7,216.
- McKay LF et al. (1981). Gum arabic metabolism in the at colon. Proceedings of the Nutrition Society 40, 73A.
- McLean Ross AH et al. (1984). A study of the effects of dietary gum arabic in the rat.British Journal of Nutrition 51,47.
- Melnick RL et al. (1983). Chronic effects of agar, guar gum, gum arabic, locust-bean gum, or tara gum in F344 rats and B6C3F1 mice.Food and Chemical Toxicology 21, 305.
- Menzies AR et al. (1996). A comparison of the physicochemical and immunological properties of the plant gum exudates of Acacia senegal (gum arabic) and Acacia seyal (gum tahla).Food Additives and Contaminants 13, 991.
- MRC Human Nutrition Research Unit (2008). Summary of narrative synthesis of health effects of potential dietary fibre components - 13th October 2008. Internet (available at: https://assets.publishing.service.gov.uk/media/5a7597d840f0b67b3d5c7b43/SACN_Narrative_Synthesis_Dietary_Fibre.pdf
- Muldoon DF Stohs SJ (1994). Modulation of ricin toxicity in mice by biologically active substances.Journal of Applied Toxicology 14, 81.
- Muthiah R et al. (1997). Hidden allergens in medications: allergy to acacia in synthroid tablets.Journal of Allergy and Clinical Immunology 99, S492.
- Nakamura J et al. (1985). Assessment of pharmaceutical excipient-induced gastrointestinal mucosal damage in rats in vivo by measuring the permeation of phenolsulfonphthalein.Chemical & Pharmaceutical Bulletin 33, 3527.

- Nishigaki I et al. (1992). Suppressive effect of curcumin on lipid peroxidation induced in rats by carbon tetrachloride or 60Co-irradiation.Journal of Clinical Biochemistry and Nutrition 13, 23.
- Nordic Working Group on Food Toxicology and Risk Assessment (NNT) (2002). Food additives in Europe 2000 - Status of safety assessments of food additives presently permitted in the EU.Nordic Food Additive Database.TEMANORD NUMBER 2002:560
- NTP (1993). Compendium of abstracts from long-term cancer studies reported by the National Toxicology Program from 1976 to 1992.Environmental Health Perspectives 101, Suppl.1
- NTP (2006). Testing status of agents at NTP Internet (available at: https://ntp.niehs.nih.gov/
- Ortega HG et al. (2002). Fatal asthma from powdering shark cartilage and review of fatal occupational asthma literature.American Journal of Industrial Medicine 42, 50.
- Osilesi O et al. (1988). Hepatic responses to edible gums during refeeding of starved rats.Journal of Nutrition 118, 462.
- Phillips GO (1984). Gums and stabilisers for the food industry 2.Pergamon Press, Oxford and New York.
- Phillips GO (1998). Acacia gum (gum arabic): a nutritional fibre; metabolism and calorific value.Food Additives and Contaminants 15, 251.
- Pimentel LA et al. (2007). Mimosa tenuiflora as a case of malformations in ruminants in the Northeastern Brazilian semiarid rangelands.Vet. Pathol.44, 928.
- Prakash AO Mathur R (1976). Screening of Indian plants for antifertility activity.Indian Journal of Experimental Biology 14, 623.
- Prival MJ et al. (1991). Bacterial mutagenicity testing of 49 food ingredients gives very few positive results.Mutation Research 260, 321.
- Psquier B et al. (1996). Viscous soluble dietary fibers alter emulsification and lipolysis of triacylglycerols in duodenal medium in vitro.Journal of Nutritional Biochemistry 7, 293.
- Rall DP (1982). Gum arabic. Federal Register 47, 35347.
- Reiser S (1984). Metabolic aspects of nonstarch polysaccharides.Food Technology 38, 107.
- Romita P et al. (2018). Occupational allergic rhinitis and contact urticaria caused by Gum Arabic in a candy factory worker. Contact Dermatitis 78(6), 427-428. DOI: 10.1111/cod.12970. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29446105
- Rubinger D et al. (1978). Hypersensitivity to tablet additives in transplant recipients on prednisone.Lancetii,689.
- Salyers AA et al. (1977). Fermentation of mucin and plant polysaccharides by strains of Bacteroides from the human colon.Applied Environmental Microbiology 33, 319.
- Salyers AA et al. (1977). Fermentation of mucins and plant polysaccharides by anaerobic bacteria from the human colon.Applied Environmental Microbiology 34, 529.
- Sander I et al. (2006). Sensitization due to gum arabic (Acacia senegal): the cause of occupational allergic asthma or crossreaction to carbohydrates?International Archives of Allergy and Immunology 141, 51.(10.1159/000094182).
- SCF (1978). Emulsifiers, stabilizers, thickeners and gelling agents. Reports of the Scientific Commission for Food. Seventh series. Commission of the European Communities.
- SCF (1995). First report on certain additives used in the manufacture of plastic materials intended to come into contact with foodstuffs. Reports of the Scientific Committee for Food (33rd series). European Commission, Luxembourg.
- SCF (1996). Opinion on additives in nutrient preparations for use in infant formulae, follow-on formulae and weaning foods. Reports of the Scientific Committee for Food (40th series).
- SCF (1997). Opinion on certain additives for use in foods for infants and young children in good health and in foods for special medical purposes for infants and young children (expressed on 21 March 1997 and amended on 13 June 1997).Annex II to minutes of the 107th Meeting, pp.25.

- SCF (1997). Opinion on certain additives for use in foods for infants and young children in good health and in foods for special medical purposes for infants and young children (expressed on 21 March 1997). Summary of recommendations. Annex V to Document III/5157/97; CS/ADD/MsAd/158-FINAL-SUMMARY, pp.3.
- Schley PD Field CJ (2002). The immune-enhancing effects of dietary fibres and prebiotics. British Journal of Nutrition 87,Suppl.2 S221.
- Schmitt D et al. (2008). Toxicologic evaluation of modified gum acacia: Mutagenicity, acute and subchronic toxicity.Food and Chemical Toxicology 46, 1048.
- Sharma RD (1984). Hypocholesterolemic activity of some Indian gums.Nutrition Research 4, 381.
- Sheu CW et al. (1985). Dominant lethal and heritable translocation tests of ammoniated glycyrhhizin, butylated hydroxytoluene and gum arabic. Journal of the American College of Toxicology 4, 101.(1985). America College of Toxicology Meeting. Food Chemical News 27, 43.
- Sheu CW et al. (1986). Tests for mutagenic effects of ammoniated glycyrrhizin, butylated hydroxytoluene, and gum arabic in rodent germ cells. Environmental Mutagenesis 8, 357.
- Shinnick FL et al. (1991). Serum cholesterol reduction by oats and other fiber sources. Cereal Food World 36,815.
- Shirasu Y et al. (1977). Mutagenicity screening on pesticides and modification products: a basis of carcinogenicity evaluation. Cold Spring Harbor Conference on Cell Proliferation 4, 267.
- Slavin J et al. (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
- Smith AG (1989). Skin disease in the pottery industry. Annals of Occupational Hygiene 33, 365.
- Smith RL et al. (2005). GRAS flavoring substances 22.Food Technology 59, 24.
- Spielman AD Baldwin HS (1933). Atopy to acacia (gum arabic). Journal of the American Medical Association 101, 444.
- Spott DA Shelley WB (1970). Exanthem due to contact allergen (benzoin) absorbed through skin.Journal of the American Medical Association 214, 1881.
- Stec J et al. (1992). Inhibition of DNA synthesis and decreased reproductive capacity in cattle maintained on semi-arid rangeland with acacia species as a major component of available browse.Veterinary and Human Toxicology 34,43.
- Steering Group on Chemical Aspects of Food Surveillance (1993). Dietary intake of food additives in the UK: initial surveillance.Food Surveillance Paper 37
- Strobel S et al. (1982). Immunogenicity of foods and food additives - in vivo testing of gums arabic, karaya and tragacanth.Toxicology Letters 14, 247.
- Strobel S et al. (1986). Immunogenicity, immunological cross reactivity and non-specific irritant properties of the exudate gums, arabic, karaya and tragacanth.Food Additives and Contaminants 3, 47.
- Strobel S Ferguson A (1986). Induction of oral tolerance, in mice, to gum arabic.Food Additives and Contaminants 3,43.
- Studdiford WE (1937). Severe and fatal reactions following the intravenous use of gum acacia glucose infusions.Surgery, Gynecology and Obstetrics 64, 772.
- Sunvold GD et al. (1995). Alteration of the fiber and lipid components of a defined-formula diet: effects on stool characteristics, nutrient digestibility, mineral balance, and energy metabolism in humans.American Journal of Clinical Nutrition 62, 1252.
- Taylor SL Hefle SL (2001). Ingredient and labeling issues associated with allergenic foods.Allergy 56, Suppl.6764.
- Thomson KF Wilkinson SM (2000). Allergic contact dermatitis to plant extracts in patients with cosmetic dermatitis.British Journal of Dermatology 142, 84.

- Titgemeyer EC et al. (1991). Fermentability of various fiber sources by human fecal bacteria in vitro.American Journal of Clinical Nutrition 53, 1418.
- Tobacman JK et al. (2000). Association between consumption of carrageenan and other gums used as food additives and incidence of mammary carcinoma in the US during the twentieth century.Proceedings of the American Association for Cancer Research 41, 92.
- Tobacman JK et al. (2001). Consumption of carrageenan and other water-soluble polymers used as food additives and incidence of mammary carcinoma.Medical Hypotheses 56, 589.
- Tomlin J (1987). Which fibre is best for the colon?Scand. J. Gastroent.22, Suppl.129 100.
- Torre M et al. (1991). Effect of dietary fiber and phytic acid on mineral availability.CRC Critical Reviews in Food Science and Nutrition 30, 1.
- Tsai AC et al. (1976). Influence of certain dietary fibers on serum and tissue cholesterol levels in rats.Journal of Nutrition 106, 118.
- Tulung B et al. (1987). Specific effects of guar gum or gum arabic on adaptation of cecal digestion to high fiber diets in the rat.Journal of Nutrition 117, 1556.
- Uhlenhuth P Remy E (1933). The question of antibodies against carbohydrate (gum arabic).Zeitschrift f-++r Immunitatsforschung und Experimentelle Therapie 79, 318.
- van Ketel WG (1984). Simultaneous sensitization to gum arabic and cobalt.Contact Dermatitis 10, 180.
- Viinanen A et al. (2011). Gum arabic as a cause of occupational allergy.Journal of Allergy 2011, 1.(10.1155/2011/841508).
- Vohra P et al. (1979). Growth inhibitory effect of some gums and pectin for Tribolium castaneum larvae, chickens and Japanese quail.Nutrition Reports International 19, 463.
- Walker R (1988). Caecal enlargement. Role of the Gut Flora in Toxicity and Cancer.Chapter 16, P383.Academic Press Ltd.
- Walter DJ et al. (1986). An experimental design to study colonic fibre fermentation in the rat: the duration of feeding.British Journal of Nutrition 55, 465.
- Walter DJ et al. (1988). Fermentation of wheat bran and gum arabic in rats fed on an elemental diet.British Journal of Nutrition 60, 225.
- Warszawska Fabryka Madla i Kosmetykow (1964).Cosmetic for skin.Polish Patent 51,517
- WHO (1974). Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers and thickening agents.WHO Food Additive Series 5, 315.
- Working Party on Food Additives (1998). Review of current research projects. Papers presented to the Working Party at its meeting on 21 October 1997, pp.22.
- Working Party on Food Additives (1999). Review of current research projects. Papers presented to the Working Party at their meeting on 13October 1998, 32 pp.
- Wyatt GM et al. (1986). A change in human faecal flora in response to inclusion of gum arabic in the diet.British Journal of Nutrition 55, 261.
- Yamaguchi T (1992). Inhibitory activity of heat treated vegetables and indigestible polysaccharides on mutagenicity.Mutation Research 284, 205.
- Zee JA et al. (1990). Effect of dietary fibers on chromium availability.Microbiologie, Aliments, Nutrition 8, 181.
- Zeiger E et al. (1992). Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals.Environmental and Molecular Mutagenesis 19, Suppl.21 2.

13. Last audited

January 2025

ARABIC GUM (Gum Arabic)

Explanation

This substance was last evaluated for acceptable daily intake for man by the Joint FAO/WHO Expert Committee on Food Additives in 1969 (see Annex I, Ref. 19). A toxicological monograph was issued in 1970 (see Annex I, Ref. 20).

Additional data have become available and are summarized and discussed in the following monograph. The previous monograph has been expanded and is reproduced in its entirety below.

BIOLOGICAL DATA

BIOLOGICAL ASPECTS

Absorption and metabolism

Based on food and caloric intake, arabic gum fed to weanling male Sprague-Dawley rats at dietary levels of 0.5 and 2.0 g/day was reported to have caloric values of 131% and 110% of corn starch, respectively (Nees, 1965).

Arabic gum is almost completely digested by guinea-pigs (O'Dell et al., 1957).

At dietary levels of less than 10%, arabic gum is fully absorbed with a caloric equivalent of 4 calories per gram (Shue et al., 1962).

Effects on enzymes and other biochemical parameters

Groups of female ZUR:SIV-Z strain rats, initial body weight 100-110 g, were gavaged twice daily, 5 days per week for 4 weeks with 20, 40 or 200 mg/kg of arabic gum as an aqueous suspension. Groups of 4 rats were killed at various time intervals and oxidative phosphorylation in isolated liver and heart mitochondria was assayed as well as mixed function oxidase activity in liver endoplasmic reticulum. A dose-dependent uncoupling of oxidative phosphorylation in heart and liver mitochondria was reported in the arabic gum groups. significant reductions occurred within 2 days to 2 weeks depending on the dose. As the feeding of arabic gum continued, liver mitochondria isolated from test animals appeared to recover. Recovery did not occur in the isolated heart mitochondria at the mid-dosage level. At the high-dose level as feeding of test animals continued, there was partial recovery in the isolated heart mitochondria followed by

another decline. The mid- and high-dose levels of arabic gum were reported to cause a progressive decline in hepatic mixed function oxidase activity, while no effect was noted at the low dose (Bachman et al., 1978).

Another study similar to the preceding one was carried out in female rats (ZUR:SIV-Z strain), golden hamsters (ZUR:LAK-Z strain), and mice (ZUR;ICR-Z strain). In mice and hamsters, there was an initial decrease in hepatic biphenyl hydroxylation; however, enzyme activity returned towards control values as the experiment progressed. In the rat, biphenyl hydroxylase activity went down and did not recover. Hepatic cytochrome b_5 and P_{450} levels were also measured; levels of these proteins were not affected by arabic gum dosing in the rat. However, cytochrome b_5 levels were depressed by the treatment in hamsters, while in mice there was a transient decrease in both cytochrome P_{450} and b_5 levels (Bachman & Zbinden, 1978).

Female rats (ZUR:SIV-Z strain) dosed by gavage with 200 mg/kg of gum arabic twice daily for 3 or 4 days showed an inhibition of phenobarbitol-stimulated aminopyrine demethylation as compared to animals not receiving gum arabic (Lutz et al., 1978).

Groups of 10 Sprague-Dawley rats received 0 or 5% of arabic gum in a 0.2% cholesterol-supplemented diet. A tracer dose of ^{14}C-labelled cholesterol was included in the animals' last meal. The animals fed arabic gum had significantly lower absorption of labelled cholesterol, although there was no effect on total carcass, serum or liver concentrations of cholesterol. However, uptake of ^{14}C-labelled cholesterol in liver and carcass was reduced. Arabic gum was associated with an increase in total cholesterol biosynthesis (Kelly & Tsai, 1978).

TOXICOLOGICAL STUDIES

Special studies on carcinogenicity

Mouse

Groups of 50 male and 50 female B6C3F1 mice were given arabic gum in the diet at concentrations of 0, 25 000 or 50 000 ppm (0, 2.5 or 5.0%) for 103 weeks. The animals were maintained on a control diet for an additional 2 weeks prior to sacrifice. No effect of the test compound on body weight gain was noted in either sex, although mean daily food consumption was reduced in both sexes of the groups receiving arabic gum. No effect of the test compound was noted with respect to survival, clinical signs, or incidence of gross or microscopic non-neoplastic lesions. Hepatocellular adenoma of the

liver was found in 2/49 controls, 0/50 low-dose and 6/49 high-dose females. Hepatocellular carcinomas were found in 1/49 controls, 2/50 low-dose and 6/50 high-dose females. The number of female mice with hepatocellular adenoma, carcinoma or unspecified neoplasm of the liver were 4/49, 2/50 and 10/49 in controls, low-dose and high-dose animals, respectively. Some high-dose animals had both a hepatocellular adenoma and a hepatocellular carcinoma. The historical records at the performing laboratory indicate the incidence of control female B6C3F1

mice with adenomas or carcinomas of the liver has been 56/975 (5.7%) with a range of 1/50 to 11/54 (2-20.3%). Male mice given arabic gum did not have an increased incidence of liver tumours. The incidence of mice with haemangiomas or haemangiosarcomas of the circulatory system was not significant in either sex. The conclusion of the study was that there was no site at which an increase in tumour incidence could be clearly associated with the administration of the chemical (National Toxicology Program, 1980).

Rat

Groups of 50 male and 50 female Fischer 344 rats were given gum arabic in the diet at concentrations of 0, 25 000 or 50 000 ppm (0, 2.5 or 5.0%) for 103 weeks. The animals were maintained on the control diets for an additional 2 weeks prior to sacrifice. In the males, body weights of test and control animals were comparable throughout the study, while in the females, weight gain in the test animals was slightly less than that of controls. The effect was not dose related. As compared to controls, feed intake was reduced in test males and test females. No effects of the test compound were reported with respect to clinical signs, survival, or incidence of gross or microscopic lesions (National Toxicology Program, 1980).

Special studies on mutagenicity

Gum arabic did not produce a measurable mutagenic response or alteration in the recombination frequency for Saccharomyces cerevisiae in either the host-mediated assay or in vitro. Similarly, no mutagenicity was reported with gum arabic either in the host-mediated assay or in vitro using Salmonella strains G-46 or TA-1530.

Cultures of bone marrow metaphase chromosomes taken from rats dosed in vivo with 50 mg or 2.5 g/kg of arabic gum showed an increased incidence of chromosomal breaks occurring within 6 hours of treatment. Similar effects were found in vitro with human WI-38 embryonic lung cells.

Gum arabic was tested using the dominant lethal gene test in

Sprague-Dawley rats. Males were given 0, 30, 2500 or 5000 mg/kg by gavage in a water suspension. A significant increase in dead implants was noted in pregnant females mated to males given a single dose of 5000 mg/kg at the third week of the study. No other significant effects were recorded and arabic gum was considered not to be a mutagen in this study (Newell & Maxwell, 1972).

No genetic activity was noted in in vitro mutagenic tests with Saccharomyces cerevisiae strain D4 and Salmonella typhimurium strains TA-1535, TA-1537 and TA-1538. Both suspension and plate test were used, with and without activation. Activation systems were prepared from liver, lung, kidney and testes from male mice, rats and monkeys (Brusick, 1975).

Gum arabic was concluded to be not mutagenic based on the sex-linked dominant lethal test in Drosophila (Valencia & Abrahamson).

Special observations on sensitivity

Sensitivity to arabic gum was found to be a true antigen-antibody response in the guinea-pig (Rice, 1955; Silvette et al., 1955).

Special studies in teratology

Groups of 21-24 pregnant Wistar-derived rats were dosed by gavage on days 6 through 15 of gestation with 0, 16, 75, 350 or 1600 mg/kg of arabic gum suspended in corn oil. No compound-related effect was observed on nidation, maternal or foetal survival, or on the incidence of hard or soft tissue anomalies occurring in the offspring. The average foetal weight at birth was slightly depressed in the high-dose group.

Groups of 19-21 pregnant CD-1 mice were dosed by gavage on days 6 through 15 of gestation with 0, 16, 75, 350 or 1600 mg/kg of arabic gum suspended in corn oil. No compound-related effect was observed on nidation, maternal or foetal survival or on the incidence of hard or soft tissue anomalies occurring in the offspring. The average foetal weight at birth was slightly depressed in the high-dose group.

Groups of 19-21 pregnant outbred golden hamsters were dosed by gavage on days 6 through 10 of gestation with 0, 16, 75, 350 or 1600 mg/kg of arabic gum suspended in corn oil. No compound-related effect was observed on nidation, maternal or foetal survival or on the incidence of hard or soft tissue anomalies occurring in the offspring.

Groups of 12-14 pregnant Dutch-belted rabbits were dosed by gavage with 0, 8, 37, 173 or 800 mg/kg of arabic gum suspended in corn oil on days 6 through 18 of gestation. The administration of up to

37 mg/kg of the test material as a suspension in anhydrous corn oil had no clear effect on nidation or on maternal or foetal survival. The number and type of abnormalities seen in foetal soft or skeletal tissues derived from this group of does did not differ from the number occurring spontaneously in the sham-treated controls. However, in 2 groups of dams dosed at 173 and 800 mg/kg bw respectively, maternal toxicity ensued with the loss of a majority of animals in the 800 mg/kg group. Death was preceded by severe bloody diarrhoea, urinary incontinence, with anorexia for 48-72 hours terminally. At

autopsy no gross pathological findings were seen other than haemorrhage in the mucosa of the small intestines. Does which survived the highest dose and bore living young to term remained outwardly normal, and the offspring were likewise normal in all respects. It was concluded that this test substance was not a teratogen in the rabbit under the test conditions employed (Morgareidge, 1972).

Acute toxicity

Animal	Route	LD_{50} (g/kg bw)	Reference
Mouse	Oral	16.0	Morgareidge, 1972
Rat	Oral	18.0	Morgareidge, 1972
Hamster	Oral	16.0	Morgareidge, 1972
Rabbit	Oral	8.0	Morgareidge, 1972

Short-term studies

Mouse

Groups of 10 male and 10 female B6C3F1 mice were fed diets containing 0, 6300, 12 500, 25 000, 50 000 or 100 000 ppm (0, 0.63, 1.25, 2.5, 5.0 or 10%) of arabic gum in the diet for 13 weeks. No compound-related effects on survival or gross or microscopic pathology were noted. Final body weights and feed consumption tended to be slightly lower in the dosed animals (National Toxicology Program, 1980).

Rat

Groups of rats were fed 0 or 15% arabic gum in their diet for 62

days. A cathartic effect was observed, but weight gain, food efficiency, haematological findings and organ weights were normal (Booth et al., 1963).

Groups of 10 male and female Fischer 344 rats were fed diets containing 0, 6300, 12 500, 25 000, 50 000 or 100 000 ppm (0, 0.63, 1.25, 2.5, 5.0 or 10%) gum arabic in the diet for 13 weeks. No compound-related effects on survival or gross or microscopic pathology were noted. Feed consumption was reduced at the 2 highest doses in the males, and at all the doses in the females. Final body weights tended to be slightly lower in the dosed animals (National Toxicology Program, 1980).

Guinea-pig

Groups of 10 and 20 guinea-pigs were fed 15% powdered arabic gum for 6 weeks. Controls received no bulk food in their diet. Weight gain was improved in the test groups (Booth et al., 1949).

Rabbit

A group of 4 rabbits was given 20% arabic gum in a casein diet for 4 weeks. Weight gain improved significantly in the test groups (Hove & Herndon, 1957).

Dog

Three dogs were given 32-35 intravenous injections of acacia over a period of 76 days at a total cumulative dosage ranging from 15.7 to 47.7 g/kg. The dog on the largest dose died with an enlarged liver. Cause of death, 4 months after its last injection, was not explanable. The other two dogs remained in good condition; biopsy showed acacia present in their livers 26 months after their last injections (Smalley et al., 1945).

Man

Nine patients with nephrotic oedema received 1-6 intravenous injections of acacia over periods up to 8 weeks, with total doses ranging from 80 to 325 g. There were no signs or symptoms of liver enlargement, and no other complications. Five of these patients excreted in the urine 5.5-38% of a single dose during periods ranging from 10 to 30 days, respectively (Johnson & Newman, 1945).

OBSERVATIONS IN MAN

Sensitivity reactions have been reported in man, e.g., asthma in printers (Brown & Crepea, 1947; Bohner et al., 1941; Sprague, 1942;

Fowler, 1952).

Occupational exposure to arabic gum has been associated with rhinitis and asthma in sensitive individuals (Cuthbert, 1973).

Sensitivity to arabic gum as a tablet additive has been reported in some kidney transplant patients. Hypersensitivity manifested as itching, and rash with fever and arthralgia was also reported in individual patients (Rubinger et al. 1978). Sensitivity of some individuals to gum arabic in food has also been reported (Gelfand, 1949).

Comments

Metabolic studies are limited, but it has been demonstrated that in the rat this gum is completely metabolized when it comprises less than 10% of the diet. Mitochondrial preparations isolated from the liver or hearts of rats maintained on diets containing arabic gum showed a dose- and time-dependent uncoupling of oxidative phosphorylation. Hepatic mixed function oxidase activity was also depressed. Changes were also reported in hepatic cytochrome b_5 and P_{450} levels in the liver of test animals maintained on diets containing arabic gum. The significance of these effects is not known, since inclusion of arabic gum in the diet of test animals at levels that cause these effects does not cause a reduction in weight gain, which would be expected if uncoupling of oxidative phosphorylation occurred.

Teratology studies were negative in rats, mice, hamsters and rabbits, although maternal toxicity was observed at very high dose levels in the rabbit.

Mutagenic studies in a number of systems, including host-mediated assay, the Ames test, Saccharomyces cerevisiae, dominant lethal test and Drosophila, were negative. Lifetime feeding studies in the rat and mouse at 5% of the diet showed no significant adverse effects.

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

Bachman, E. et al. (1978) Biochemical effects of gum arabic gum, tragacanth, methylcellulose and carboxymethylcellulose in the rat heart and liver, Pharmacol., 17, 39-49

Bachman, E. & Zbinden, G. (1978) Biochemical effects of chronically administered suspended agents on mitochondrial metabolism and hepatic mixed function oxidases in rat, mice and hamster, Arch. Toxicol. Suppl., 1, 183-187

Bohner, C. B., Sheldon, J. M. & Trenis, J. W. (1941) Sensitivity of gum acacia with a report of ten cases of asthma in printers, J. Allergy, 12, 290

Booth, A. N., Elvehjem, C. A. & Hart, E. B. (1949) The importance of bulk in the nutrition of the guinea pig, J. Nutr., 37, 263

Booth, A. N., Hendrickson, A. P. & De Eds, F. (1963) Physiologic effects of three microbial polysaccharides on rats, Toxicol. Appl. Pharmacol., 5, 478

Brown, E. B. & Crepea, S. B. (1947) Allergy (asthma) to ingested gum tragacanth - a case report, J. Allergy, 18, 214

Brusick, E. (1975) Mutagenic evaluation of compound FDA 71-15, gum arabic. Unpublished. Litton Bionetics, Inc. Contract, 32 pp. Contract No. 223-74-2104. Submitted to the World Health Organization by the US Food and Drug Administration

Cuthbert, O. D. (1973) Investigation into an outbreak of rhinitis and asthma in a printing works, Ann. Occup. Hyg., 16, 203

Fowler, P. B. S. (1952) Printer's asthma, Lancet, 2, 755

Gelfand, H. H. (1949) The vegetable gums by ingestion in the etiology of allergic disorders, J. Allergy, 20, 311-321

Hove, E. L. & Herndon, F. J. (1957) Growth of rabbits on purified diets, J. Nutr., 63, 193

Johnson, J. B. & Newman, L. H. (1945) Intravenous injection of acacia, Arch. Intern. Med., 76, 167

Kelly, J. & Tsai, A. (1978) Effects of pectin, gum arabic and agar on cholesterol absorption, synthesis and turnover in rats, J. Nutr., 108, 630-639

Lutz, W. K., Braudle, E. & Zbinden, G. (1978) Effect of gum arabic on aminopyrine demethylation in rats, Experientia, 34, 1609-1610

Newell, G. W. & Maxwell, W. A. (1972) Study of mutagenic effects of gum arabic. Unpublished. Standard Research Institute, pp. 31. Contract No. FDA 71-267. Submitted to the World Health Organization by the US Food and Drug Administration

Morgareidge, K. (1972a) Acute toxicity studies on gum arabic. Unpublished. Food and Drug Research Laboratories, Inc. Contract No. FDA 71-260. Submitted to the World Health Organization by the US Food and Drug Administration

Morgareidge, K. (1972b) Teratologic evaluation of FDA 71-15 (gum arabic). Unpublished. Food and Drug Research Laboratories, Inc. Contract No. FDA 71-260. Submitted to the World Health Organization by the US Food and Drug Administration

National Toxicology Program (1980) Carcinogenesis bioassay of gum arabic. Unpublished. National Toxicology Program, Research Triangle Park, North Carolina, pp. 134. Submitted to the World Health Organization by the US Food and Drug Administration

Nees, P. W. (1965) Hallmark pure gum arabic No. 1 U.S.P. - evaluation of caloric content. Unpublished. In: Wisconsin Alumni Foundation, Madison, Wisconsin, pp. 3, (Assay Report No. 4121036. Submitted to the World Health Organization by the US Food and Drug Administration

O'Dell, B. L. et al. (1957) Diet composition and mineral balance in guinea pigs, J. Nutr., 63, 65

Rice, C. E. (1955) An investigation of some of the factors determining the decrease in complement activity in anaphylactic shock, J. Immunol., 75, 85-95

Rubinger, D., Friedlander, M. & Superstine, E. (1978) Hypersensitivity to tablet additives transplant recipients on prednisone, Lancet, 2, 689

Silvette, M., Swinford, O., Jr & Tull, L. (1955) Observations on acacia as an immunizing, sensitizing anaphylactogenic and desensitizing antigen, J. Allergy, 26, 509-518

Shue, G. M., Douglass, C. D. & Friedman, L. (1962) Nutritional studies of complex carbohydrates, Fed. Proc., 21(2), 91

Smalley, R. E. et al. (1945) Effect of intravenously administered solution of acacia on animals, Arch. Intern. Med., 76, 39

Sprague, P. H. (1942) Bronchial asthma due to sensitivity to gum acacia, Canad. Med. Ass. J., 47, 253

Valencia, R. & Abrahamson, S. (undated) Drosophila sex-linked recessive lethal test on gum arabic. Unpublished. Contract No. FDA 223-77-2119, pp. 4. Dept. of Zoology, University of Wisconsin. Submitted to the World Health Organization by the US Food and Drug Administration

See Also:

Toxicological Abbreviations

Arabic gum (FAO Nutrition Meetings Report Series 46a)

Arabic gum (WHO Food Additives Series 5)

NTP Technical Report

on the

CARCINOGENESIS BIOASSAY

of

GUM ARABIC

(CAS No. 9000-01-5)

in F344 RATS AND B6C3F$_1$ MICE

(FEED STUDY)

NATIONAL TOXICOLOGY PROGRAM
Research Triangle Park
Box 12233
North Carolina 27709
and
Bethesda, Maryland 20205

May 1982

NTP-80-81
NIH Publication No. 82-1783

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

NOTE TO THE READER

This is one in a series of experiments designed to determine whether selected chemicals produce cancer in animals. Chemicals selected for testing in the NTP carcinogenesis bioassay program are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection per se is not an indicator of a chemical's carcinogenic potential. Negative results, in which the test animals do not have a greater incidence of cancer than control animals, do not necessarily mean that a test chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a test chemical is carcinogenic for animals under the conditions of the test and indicate that exposure to the chemical is a potential hazard to humans. The determination of the risk to humans from chemicals found to be carcinogenic in animals requires a wider analysis which extends beyond the purview of this study.

This study was initiated by the National Cancer Institute's Carcinogenesis Testing Program, now part of the National Institute of Environmental Health Sciences, National Toxicology Program.

Comments and questions about the National Toxicology Program Technical Reports on Carcinogenesis Bioassays should be directed to the National Toxicology Program, located at Room A-306, Landow Building, Bethesda, MD 20205 (301-496-1152) or at Research Triangle Park, NC 27709 (919-541-3991).

Although every effort is made to prepare the Technical Reports as accurately as possible, mistakes may occur. Readers are requested to communicate any mistakes to the Deputy Director, NTP (P.O. Box 12233, Research Triangle Park, NC 27709), so that corrective action may be taken. Further, anyone who is aware of related ongoing or published studies not mentioned in ths report is encouraged to make this information known to the NTP.

These NTP Technical Reports are available for sale from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161 (703-487-4650).

Single copies of this carcinogenesis bioassay technical report are available without charge (and while supplies last) from the NTP Public Information Office, National Toxicology Program, P.O. Box 12233, Research Triangle Park, NC 27709.

TABLE OF CONTENTS

TABLES

APPENDIXES

ABSTRACT

A carcinogenesis bioassay of gum arabic (81-86% pure), a widely used food stabilizer, was conducted by feeding diets containing 25,000 or 50,000 ppm of the test substance to 50 F344 rats and 50 B6C3F1 mice of each sex for 103 weeks. Groups of untreated rats and mice of each sex served as controls.

Throughout most of the study, mean body weights of dosed male and female mice and of dosed male rats were comparable with those of the controls; mean body weights of the dosed female rats were slightly lower than those of the controls. No other compound-related clinical signs or effects on survival were observed. Mean daily feed consumption by high-dose rats and mice of either sex was 85% to 94% that of the controls. The high dose (50,000 ppm) used in this bioassay is the maximum concentration (5%) currently used in feed studies.

Statistically significant ($P<0.05$) increasing trends were observed for the number of female mice with heptocellular carcinomas (1/49, 2/50, 6/50), and with total liver tumors (4/49, 2/50, 10/50). No statistically significant differences were obtained when comparing the control rates with those observed in the treated groups. These observations were not considered to be clearly associated with the dietary administration of gum arabic. Thus, no compound-related neoplastic or nonneoplastic lesions were found in rats or mice of either sex.

Under the conditions of this bioassay, gum arabic was not carcinogenic for F344 rats or B6C3F1 mice of either sex.

CONTRIBUTORS

The bioassay of gum arabic was conducted at EG&G Mason Research Institute, Worcester, Massachusetts, under a subcontract to Tracor Jitco, Inc., Rockville, Maryland, prime contractor for the NCI Carcinogenesis Testing Program. The 2-year study in mice was initiated in June 1977 and completed in June 1979, and the 2-year study in rats was begun in July 1977 and finished in July 1979.

The bioassay was conducted under the supervision of Drs. H. Lilja (1) and E. Massaro (1,2), principal investigators. Doses of the test chemical were selected by Drs. J. Robens (3,4) and C. Cueto (5). The program manager was Ms. R. Monson (1). Ms. A. Good (1) supervised the technicians in charge of animal care, and Ms. E. Zepp (1) supervised the preparation of the feed mixtures and collected samples of the diets for analysis. Ms. D. Bouthot (1) kept all daily records of the test. Dr. A. Russfield (1), pathologist, directed the necropsies and performed the histopathologic evaluations. The pathology report and selected slides were evaluated by the NCI Pathology Working Group as described in Ward et al. (1978). The diagnoses represent a consensus of contracting pathologists and the NCI Pathology Working Group, with final approval by the NCI Pathology Working Group, which consisted of: G. Reznik (6), J. Ward (6), and P. Hildebrandt (3) who met on August 11, 1980.

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute, Rockville, Maryland (7). The statistical analyses were performed by Dr. J. R. Joiner (3) and Mr. J. Warner (3), using methods selected for the bioassay program by Dr. J. J. Gart (8). Chemicals used in this bioassay were analyzed at Midwest Research Institute (9).

This report was prepared at Tracor Jitco (3). Those responsible for the report at Tracor Jitco were Dr. C. Cueto (5), Director of the Bioassay Program; Dr. S. S. Olin, Associate Director; Dr. M. A. Stedham, pathologist; Dr. J. E. Tomaszewski, chemist; Dr. W. D. Theriault, reports manager; and Dr. A. C. Jacobs, bioscience writer.

The following scientists at NCI/NTP (6) were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. J. Fielding Douglas, Dr. Charles Grieshaber, Dr. James Huff (chemical manager), Dr. Joseph Haseman, Dr. Larry Hart, Dr. Ernest E. McConnell, Dr. John A. Moore, Dr. Sherman F. Stinson, Dr. R. Tennant, and Dr. Jerrold M. Ward.

---

(1) EG&G Mason Research Institute, 57 Union Street, Worcester, Massachusetts 06108.

(2) Now with Pennsylvania State University, 226 Fenske Laboratory, University Park, Pennsylvania 16802.

(3) Tracor Jitco, Inc., 1776 East Jefferson Street, Rockville, Maryland 20852.

(4) Now with Bureau of Veterinary Medicine, Food and Drug Administration, 5600 Fishers Lane, Rockville, Maryland 20857.

(5) Now with Clement Associates, 1010 Wisconsin Avenue, N.W., Washington, D.C. 20007.

(6) Bioassay Program, National Institutes of Health, Bethesda, Maryland 20205; National Toxicology Program, Research Triangle Park, Box 12233, North Carolina 27709.

(7) EG&G Mason Research Institute, 1530 East Jefferson Street, Rockville, Maryland 20852.

(8) Mathematical Statistics and Applied Mathematics Section, Biometry Branch, Field Studies and Statistics, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205.

(9) Midwest Research Institute, 425 Volker Boulevard, Kansas City, Missouri 64110.

SUMMARY OF PEER REVIEW COMMENTS

On February 18, 1981 this carcinogenesis bioassay report on gum arabic underwent peer review and was approved by the National Toxicology Program Board of Scientific Counselors' Technical Report Review Subcommittee and associated Panel of Experts at an open meeting held in Room 31C, National Institutes of Health, Bethesda, Maryland. Members of the Subcommittee are Drs. Margaret Hitchcock (Chairperson), Curtis Harper, and Alice Whittemore. Members of the Panel are Drs. Normal Breslow, Joseph Highland, Frank Mirer, Sheldon Murphy (a principal reviewer), Svend Nielsen, Bernard Schwetz, Roy Shore, James Swenberg, and Gary Williams (a second principal reviewer). Drs. Breslow and Whittemore were unable to attend this meeting.

Dr. Murphy, a principal reviewer for the report on the carcinogenesis bioassay of gum arabic, agreed with the conclusion for a lack of carcinogenic action in rats and mice. He noted an increase in the number of hepatocellular adenomas or carcinomas in high-dose female mice which was not statistically significant by the pairwise comparison test, but was significant for a positive linear trend. These effects should not be regarded as compound related. There was a significantly decreased incidence of malignant lymphomas in high-dose male rats; yet this observation was diminished completely when the combined incidence of leukemias or lymphomas was evaluated.

As the second principal reviewer, Dr. Williams concurred with Dr. Murphy's review. Dr. Mirer said he would have liked to have seen more information included on what impurities and low molecular weight materials are found in gum arabic. Dr. Schwetz said that, as with the other food additives, the summary should clearly identify 50,000 ppm as a maximum concentration for feeding studies.

Dr. Murphy moved that the report on the bioassay of gum arabic be accepted and that statements be included to indicate 50,000 ppm as the maximum allowable concentration for feeding studies, and that the occurrence of an increase in hepatocellular neoplasms, carcinomas, or adenomas in high-dose female mice was not associated with gum arabic feeding. Dr. Williams seconded the motion, and the report was approved unanimously by the Peer Review Panel.

I. INTRODUCTION

Gum arabic (CAS No. 9000-01-5), also known as gum acacia, is the dried exudate from the branches of various species of Acacia. The major source of gum arabic used in the United States is Acacia Senegal from the Republic of Sudan (Furia, 1972). In 1971, 30 million pounds were imported into the United States (Life Sciences Research Office, 1973).

Structurally, gum arabic is a neutral or slightly acidic salt of a complex polysaccharide composed of galactose, arabinose, rhamnose, glucuronic acid, 4-O-methylglucuronic acid, calcium, magnesium, and potassium. The molecular weight has been reported to be 600,000 (Anderson and Dea, 1971). Gum arabic is distinguished from other gums by its high solubility in water; 50% solutions can be prepared, compared with maximum concentrations of 5% or less for most other gums (Furia, 1972).

Gum arabic is approved for use as a food additive by the U. S. Food and Drug Administration and is on the list of substances "generally recognized as safe" (CFR, 1974). Gum acacia is used as a flavor fixative in dry packaged food mixes, a foam stabilizer in soft drinks and beer, an adhesive for icings and toppings, and an emulsifier and stabilizer in confectionaries (Furia, 1972).

The following products may contain gum arabic at approximately the concentrations indicated: candy (28%); chewing gum (2.8%); imitation dairy products, frostings, fats and oils, and grain products (1%); sugar substitutes, fruit ices, nut products, and gelatin puddings (0.5% - 0.06%); baked goods, meat products, and alcoholic beverages (0.15% - 0.06%); instant coffee and tea (0.08% - 0.01%); nonalcoholic beverages (0.06% - 0.04%), processed fruit, frozen dairy products, and breakfast cereals (0.02% - 0.007%) (Life Sciences Research Office, 1973).

Gum arabic is used as an excipient for pills and tablets, a syrup for the suspension of insoluble drugs, an emulsion stabilizer for lotions and protective creams, and a pigment binder in face powders and rouges (Kirk and Othmer, 1966).

Gum arabic may be added to various glues, pastes, and binding cements, to paint and pigment formulations, and to inks. This gum is also used as a sizing and finishing agent in the textile industry, a corrosion inhibitor in storage batteries, and a binder for insecticides (Kirk and Othmer, 1966).

The oral LD_{50} of gum arabic in rats and mice is greater than 16 g/kg body weight (Bailey and Morgareidge, 1976).

Gum arabic was not mutagenic when tested without metabolic activation in several short-term mutagenicity assay systems, including Salmonella typhimurium TA 1530 and G-46 and Saccharomyces cerevisiae D-3. Gum arabic was not tested with metabolic activation (Green, 1977).

Gum arabic was tested by the NCI/NTP because of its widespread use as a food additive and therefore, the widespread exposure of the human population and because of the absence of carcinogenicity data.

II. MATERIALS AND METHODS

A. Chemical

Gum arabic (CAS No. 9000-01-5) was obtained in two batches from the Stein Hall Company, a division of Celanese Polymer Specialties Company (Louisville, KY). Lot No. 54-36431 was used for the subchronic studies and the first 3 months of the chronic studies. Lot No. 54-77890 was used for the rest of the chronic studies.

Purity and identity analyses were conducted at Midwest Research Institute (Appendixes E and F). Results of titration by periodate oxidation indicated that Lot No. 54-36431 was 80.8% pure and that Lot No. 54-77890 was 85.5% pure based on an assay for mannitol as compared with a glucose standard. Results of the Karl Fisher titrations indicated 12.3% water in Lot No. 5436431 and 9.0% water in Lot No. 54-77890. Four components in the hydrolysates of each batch of gum arabic were separated by thin-layer chromatography; three were identified as D-galactose, L-rhamnose, and L-arabinose. The fourth component may have been glucuronic acid. The infrared spectra of both batches were consistent with the literature spectra. The infrared spectra of both lots of gum arabic taken on a periodic basis at the bioassay laboratory showed no change over the course of the study.

B. Dietary Preparation

Each test diet was prepared by mixing the chemical and an aliquot of Wayne Lab Blox® meal with a mortar and pestle and then adding this premix to the rest of the feed and mixing in a Patterson-Kelly® twin-shell V-blender for 15 minutes. Test diets were sealed in labelled plastic bags and stored at 4°C for no longer than 14 days.

Due to some similar components in the test substance and feed, the quantitative method available could not measure concentration levels used in

the chronic study reproducibly within ±10%. Thus, formulated diets were not analyzed for concentrations of gum arabic during the study.

C. Animals

Four-week old F344 rats and B6C3F1 mice were obtained from the NCI Frederick Cancer Research Center (Frederick, MD) and observed for the presence of parasites and other diseases (8 days for rats and 9 days for mice). The animals were then randomly assigned to cages, and the cages were randomly assigned to control or dosed groups.

D. Animal Maintenance

Rats and mice were housed five per cage in suspended polycarbonate cages equipped with disposable nonwoven fiber filter sheets (Table 1). Cages and hardwood chip bedding were changed twice weekly, and cage racks were changed every 2 weeks. Water was supplied by an Edstrom automatic watering system, and Wayne Lab Blox® meal in stainless-steel, gang-style hoppers was available *ad libitum*.

The temperature in the animals rooms ranged from 19° to 32°C (average 23.8°C), and relative humidity was uncontrolled (average 43%). Incoming air was filtered through Tri-Dek 15/40 denier Dacron filters. Room air was changed 10 to 12 times per hour. Fluorescent lighting was provided 12 hours per day.

For the first 4 months of the chronic study, rats and mice were housed by species in separate rooms in which chronic feed studies were being conducted for locust bean gum (CAS No. 9000-40-2). For the remainder of the chronic study, rats and mice fed gum arabic were housed in the same room, and no other chemicals were on test in that room.

Table 1. Sources and Descriptions of Materials Used for Animal Maintenance

Item	Description	Source
Animal Feed	Wayne® Lab Blox Meal	Allied Mills (Chicago, IL)
Feed Hoppers	Stainless steel, gang style	Scientific Cages, (Bryan, TX)
Cages	Polycarbonate	Lab Products, Inc. (Garfield, NJ)
Filter Sheets	Disposable, non-woven fiber	Lab Products, Inc. (Rochelle, Park, NJ)
Bedding	Hardwood chips: Aspen bed®	American Excelsior (Baltimore, MD)
	Beta® chips	Agway Corp. (Syracuse, NY)
Cage and Hopper Washer	Adamation Cage Washer	Adamation (Newton, MA)
Rack Washer	Kleen-King Jet-Spray Washer	Britt-Tech Corp. (Britt, IA)

E. Repeated-Dose Studies

Repeated-dose feed studies were conducted using F344 rats and B6C3F1 mice to determine the concentrations of gum arabic to be used in the subchronic studies.

In the repeated dose study, groups of five males and five females of each species were fed diets containing 0, 6,300, 12,500, 25,000, 50,000, or 100,000 ppm gum arabic for 14 days. One male rat receiving 100,000 ppm died. All surviving animals were killed on day 15. No compound-related effects were observed.

F. Subchronic Studies

Subchronic studies were conducted to determine the concentrations to be used in the chronic studies. Diets containing 0, 6,300, 12,500, 25,000, 50,000, or 100,000 ppm were fed for 13 weeks to groups of 10 males and 10 females of each species. Mortality checks were made twice daily, and animals were weighed weekly (Tables 2 and 3). Feed consumption was measured during weeks 4, 8, and 12 (Table 4). At the end of the 91-day study, survivors were killed, necropsies were performed on all animals, and tissues (see Section H) were taken for histopathologic analysis.

Rats: No compound-related effects were observed, except for a reduction in feed consumption at the two highest doses in males and at all doses in females as compared with control animals.

Doses selected for the rats for the chronic study were 25,000 and 50,000 ppm gum arabic in feed, since the maximum concentration recommended for chronic feeding studies is 50,000 ppm (NCI, 1976).

Mice: No compound-related effects were observed. Doses selected for the mice for the chronic study were 25,000 and 50,000 ppm gum arabic in feed.

Table 2. Dosage, Survival, and Mean Body Weights of Rats Fed Diets Containing Gum Arabic for 13 Weeks

Dose (ppm)	Survival (a)	Mean Body Weights (grams)			Weight Change Relative to Controls (b) (Percent)
		Initial	Final	Change	
MALE					
0	10/10	85.3	350.8	+265.5	
6,300	10/10	85.1	337.4	+252.3	-5
12,500	10/10	85.5	336.1	+250.6	-6
25,000	10/10	85.9	340.4	+254.5	-4
50,000	10/10	85.4	337.5	+252.1	-5
100,000	10/10	85.6	332.4	+246.8	-7
FEMALE					
0	10/10	79.0	202.1	+123.1	
6,300	10/10	78.7	199.0	+120.3	-2
12,500	10/10	76.2	200.3	+124.1	+1
25,000	10/10	78.6	198.5	+119.9	-3
50,000	10/10	78.4	198.7	+120.3	-2
100,000	10/10	77.8	190.5	+112.7	-8

(a) Number surviving/number per group.

(b) Weight Change Relative to Controls =

$$\frac{\text{Weight Change (Dosed Group)} - \text{Weight Change (Control Group)}}{\text{Weight Change (Control Group)}} \times 100$$

Table 3. Dosage, Survival, and Mean Body Weights of Mice Fed Diets Containing Gum Arabic for 13 weeks

Dose (ppm)	Survival (a)	Mean Body Weights (grams)			Weight Change Relative to Controls (b) (Percent)
		Initial	Final	Change	
MALE					
0	10/10	20.9	34.2	+13.3	
6,300	10/10	20.9	34.3	+13.4	+1
12,500	10/10	20.9	33.1	+12.2	-8
25,000	10/10	20.9	34.2	+13.3	0
50,000	10/10	20.9	35.0	+14.1	+6
100,000	10/10	20.9	32.9	+12.0	-10
FEMALE					
0	10/10	17.9	27.6	+9.7	
6,300	10/10	18.2	26.7	+8.5	-8
12,500	10/10	18.1	25.5	+7.4	-23
25,000	10/10	17.9	25.7	+7.8	-20
50,000	10/10	17.9	28.2	+10.3	+6
100,000	10/10	17.8	25.7	+7.9	-19

(a) Number surviving/number per group.
(b) Weight Change Relative to Controls =

$$\frac{\text{Weight Change (Dosed Group)} - \text{Weight Change (Control Group)}}{\text{Weight Change (Control Group)}} \times 100$$

Table 4. Feed Consumption by Rats and Mice Fed Diets Containing 100,000 ppm Gum Arabic for 13 Weeks

Week No.	Control grams/kg (a)	Highest Dose (b) grams/kg (a)	Highest Dose/Control (c)
Male Rats			
4	718	696	1.0
8	467	503	1.1
12	507	430	0.8
Female Rats			
4	779	645	0.8
8	692	500	0.7
12	714	490	0.7
Male Mice			
4	1,956	1,650	0.8
8	1,650	1,394	0.8
12	1,245	1,253	1.0
Female Mice			
4	3,313	1,820	0.5
8	2,484	1,836	0.7
12	1,737	2,047	1.2

(a) Grams of feed consumed per kg of body weight
(b) Highest dose is 100,000 ppm
(c) Ratio of the grams/kg for the highest dose group to the grams/kg for the controls

G. Chronic Studies

The number of animals per group, the concentration of the test substance in the diet, and the duration of the chronic studies are shown in Table 5.

H. Clinical Examinations and Pathology

Mortality checks were made twice daily, and animals were weighed monthly. Animals that were moribund and those that survived to the end of the study were killed with carbon dioxide and necropsied.

Gross and microscopic examinations were performed on major tissues and major organs, and on all gross lesions from killed animals and from animals found dead unless precluded in whole or in part autolysis or cannibalization. Thus, the number of animals from which particular organs or tissues were examined microscopically varies and does not necessarily represent the number of animals that were placed on study in each group. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The following were examined microscopically: skin, lungs and bronchi, trachea, bone and bone marrow, spleen, lymph nodes, heart, salivary gland, liver, pancreas, stomach, small intestine, large intestine, kidneys, urinary bladder, pituitary, adrenal, thyroid, parathyroid, mammary gland, prostate and seminal vesicles or uterus, testis or ovary, brain, thymus, larynx, and esophagus.

I. Data Recording and Statistical Analyses

Data on this experiment were recorded in the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969).

Table 5. Experimental Design of Chronic Feeding Studies with Gum Arabic in Rats and Mice

Test Group	Initial No. of Animals	Gum Arabic (ppm)	Weeks on Study	
			Dosed	Not Dosed
Male Rats				
Control	50	0	0	105
Low-Dose	50	25,000	103	2
High-Dose	50	50,000	103	2
Female Rats				
Control	50	0	0	105
Low-Dose	50	25,000	103	2
High-Dose	50	50,000	103	2
Male Mice				
Control	50	0	0	105
Low-Dose	50	25,000	103	2
High-Dose	50	50,000	103	2
Female Mice				
Control	50	0	0	105
Low-Dose	50	25,000	103	2
High-Dose	50	50,000	103	2

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) extensions of Cox's method for testing for a dose-related trend. One-tailed P values have been reported for all tests except the departure from linearity test, which is reported only when its two-tailed P value is less than 0.05.

The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals in which that site is examined (denominator). In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions prior to histologic sampling (e.g., skin or mammary tumors) or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As part of these analyses, the one-tailed Fisher exact test (Cox, 1970) was used to compare the tumor incidence of a control group with that of a group of dosed animals at each dose level. When the results from two dosed groups are compared simultaneously with that for a control group, a correction to ensure an overall significance level of 0.05 is made. The Bonferroni inequality criterion (Miller, 1966) requires that the P values for any comparison be less than or equal to 0.025. When this correction was used, it is discussed in the narrative section. It is not presented in the tables, where the Fisher exact P values are shown.

The Cochran-Armitage test for linear trend in proportions, with continuity correction (Armitage, 1971), was also used. Under the assumption of a linear trend, this test determines if the slope of the dose-response curve is different from zero at the one-tailed 0.05 level of significance. Unless otherwise noted, the direction of the significant trend is a positive dose relationship. This method also provides a two-tailed test of departure from linear trend.

A time-adjusted analysis was applied. In this analysis, deaths that occurred before the first tumor was observed were excluded by basing the statistical tests on animals that survived at least 52 weeks, unless a tumor was found at an anatomic site of interest before week 52. When such an early tumor was found, comparisons were based exclusively on animals that survived at least as long as the animals in which the first tumor was found. Once this reduced set of data was obtained, the standard procedures for analyses of the incidence of tumors (Fisher exact tests, Cochran-Armitage tests, etc.) were followed.

Life table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an observed tumor were computed as in Saffiotti et al. (1972). The week during which an animal died naturally or was killed was entered as the time point of tumor observation. The methods of Cox and of Tarone were used for the statistical tests of the groups. The statistical tests were one-tailed.

The approximate 95% confidence interval for the relative risk of each dosed group compared with its control was calculated from the exact interval on the odds ratio (Gart, 1971).

III. RESULTS - RATS

A. Body Weights and Clinical Signs (Rats)

Mean body weights of dosed and control male rats were comparable throughout the study. The mean body weights of dosed female rats were slightly lower than those of the controls (Figure 1 and Table 6). No compound-related clinical signs were observed. The mean daily feed consumption per animal was 94% (20.8/22.1) and 88% (19.4/22.1) for low- and high-dose male rats and 88% (16.4/18.7) and 87% (16.3/18.7) for low- and high-dose female rats, compared with controls (Appendix G).

B. Survival (Rats)

Estimates of the probabilities of survival of male and female rats fed diets containing gum arabic at the concentrations of this bioassay, together with those of the control group, are shown by the Kaplan and Meier curves in Figure 2. No significant differences in survival were found between any group of rats of either sex.

In male rats, 26/50 (52%) of the controls, 26/50 (52%) of the low-dose, and 29/50 (58%) of the high-dose group lived to the end of the study at 105 weeks. In female rats, 34/50 (68%) of the controls, 36/50 (72%) of the low-dose, and 32/50 (64%) of the high-dose group lived to the end of the study at 105 weeks.

C. Pathology (Rats)

Histopathologic findings on neoplasms in rats are summarized in Appendix A, Tables A1 and A2; findings on nonneoplastic lesions are summarized in Appendix C, Tables C1 and C2.

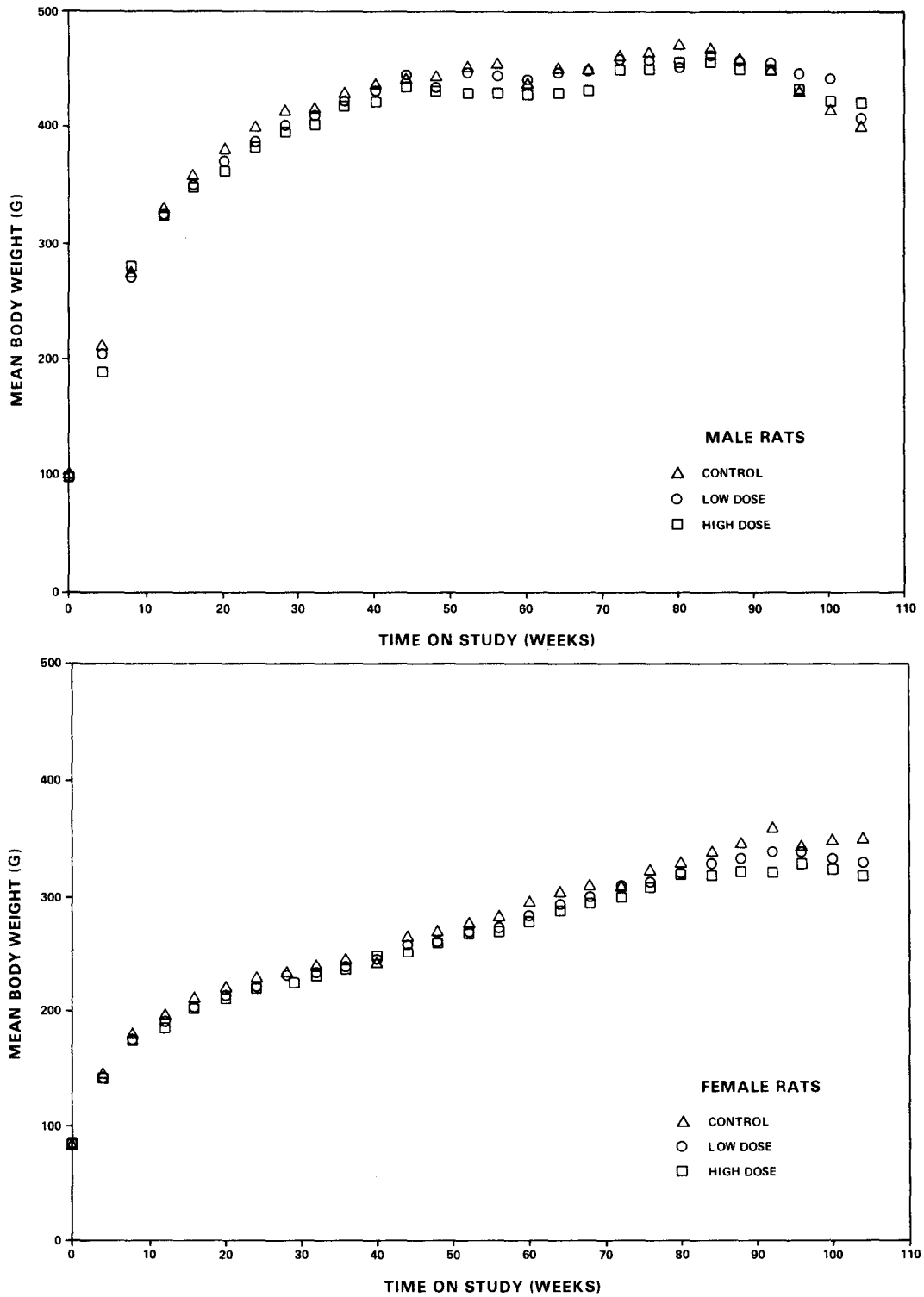

Figure 1. Growth Curves for Rats Fed Diets Containing Gum Arabic

Table 6. Mean Body Weight Change (Relative to Controls) of Rats Fed Diets Containing Gum Arabic

	Week No.	Mean Body Weight Change (grams)			Weight Change Relative to Controls (a) (Percent)	
		Control	Low Dose	High Dose	Low Dose	High Dose
Male	0	100(b)	97(b)	97(b)		
	4	113	107	90	-5	-20
	24	298	289	285	-3	-4
	44	342	348	337	+2	-1
	64	352	349	331	-1	-6
	84	367	365	359	-1	-2
	104	298	315	323	+6	+8
Female	0	84(b)	85(b)	85(b)		
	4	62	58	57	-6	-8
	24	144	136	134	-6	-7
	44	182	172	167	-5	-8
	64	221	209	202	-5	-9
	84	254	242	234	-5	-8
	104	267	236	237	-12	-11

(d) Weight Change Relative to Controls =

$$\frac{\text{Weight Change (Dosed Group)} - \text{Weight Change (Control Group)}}{\text{Weight Change (Control Group)}} \times 100$$

(b) Initial weight

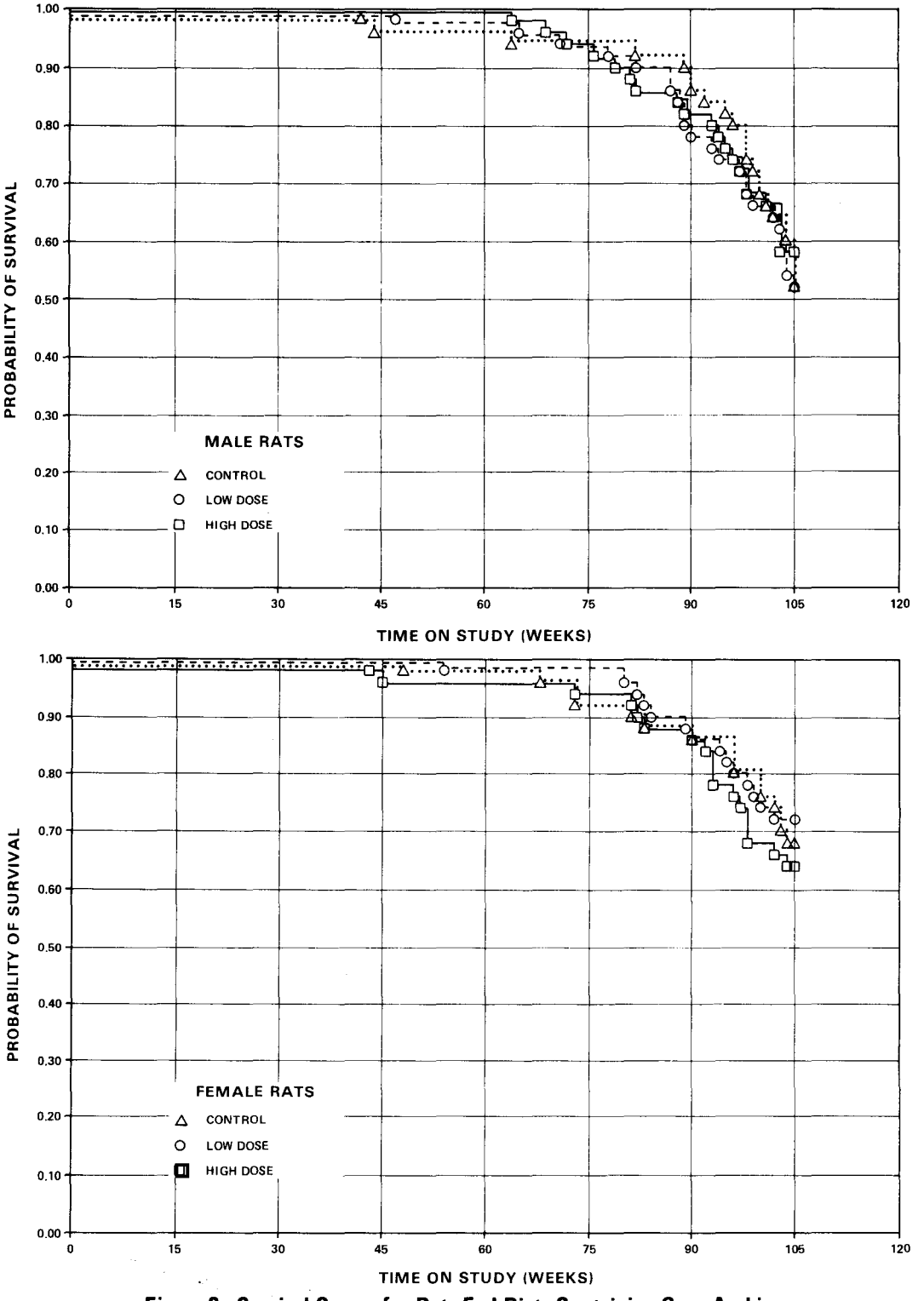

Figure 2. Survival Curves for Rats Fed Diets Containing Gum Arabic

The tumors encountered were those commonly found in aging rats of this strain. Rats in all groups exhibited a variety of nonneoplastic, inflammatory, and degenerative changes. None were considered to be associated with administration of the compound.

The results of the histopathologic examination indicated that gum arabic was not carcinogenic or toxic for F344 rats under the conditions of this bioassay.

D. Statistical Analyses of Results (Rats)

Tables 7 and 8 contain the statistical analyses of those primary tumors that met both of the following criteria: (1) at least two animals in one group had the tumor, and (2) the incidence in one or more groups was at least 5%.

Malignant lymphomas of the hematopoietic system were observed in male rats in a statistically significant negative relation (8/50, 16% in the controls; 4/50, 8% in the low-dose; and 1/50, 2% in the high-dose). The historical rate for malignant lymphomas in male control rats at EG&G Mason Laboratories is 28/834 (3.4%). The Cochran-Armitage test for linear trend was statistically significant in the negative direction (P=0.011), and the Fisher exact test between the high-dose group and the control group was significant (P=0.015). No significant incidence was observed in the low-dose group; however, this tumor occurred in decreased incidence in the low-dose group compared with the control group. In female rats, this tumor was not observed in statistically significant proportions, and no significant differences were observed in the incidence of animals with either leukemia or lymphoma.

Time adjusted analysis eliminating those animals dying before 52 weeks did not materially alter the results, since few early deaths occurred. Life table analyses, using the week of death with observed tumor, did not materially alter the results reported above.

Table 7. Analyses of the Incidence of Primary Tumors in Male Rats Fed Diets Containing Gum Arabic (a)

Topography: Morphology	Control	Low Dose	High Dose
Hematopoietic System:			
Leukemia (b)	10/50(20)	15/50(30)	14/50(28)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.500	1.400
Lower Limit		0.701	0.642
Upper Limit		3.359	3.177
Weeks to First Observed Tumor	90	88	69
Hematopoietic System:			
Malignant Lymphoma,			
Lymphocytic Leukemia (b)	2/50(4)	3/50(6)	1/50(2)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.500	0.500
Lower Limit		0.180	0.009
Upper Limit		17.329	9.290
Weeks to First Observed Tumor	98	104	98
Hematopoietic System:			
Malignant Lymphoma (b)	8/50(16)	4/50(8)	1/50(2)
P Values (c),(d)	P=0.011(N)	N.S.	P=0.015(N)
Relative Risk (Control) (e)		0.500	0.125
Lower Limit		0.117	0.003
Upper Limit		1.737	0.880
Weeks to First Observed Tumor	44	104	98

Table 7. Analyses of the Incidence of Primary Tumors in Male Rats Fed Diets Containing Gum Arabic (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Hematopoietic System:			
Leukemia or Lymphoma (b)	18/50(36)	19/50(38)	16/50(32)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.056	0.889
Lower Limit		0.601	0.483
Upper Limit		1.860	1.624
Weeks to First Observed Tumor	44	88	69
Liver: Neoplastic Nodule (b)	3/49(6)	2/50(4)	4/50(8)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.653	1.307
Lower Limit		0.057	0.233
Upper Limit		5.457	8.508
Weeks to First Observed Tumor	100	105	101
Liver: Hepatocellular Carcinoma (b)	1/49(2)	3/50(6)	1/50(2)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		2.940	0.980
Lower Limit		0.246	0.013
Upper Limit		151.180	75.404
Weeks to First Observed Tumor	96	97	105

Table 7. Analyses of the Incidence of Primary Tumors in Male Rats Fed Diets Containing Gum Arabic (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Liver: Neoplastic Nodule or Hepatocellular Carcinoma (b)	4/49(8)	5/50(10)	5/50(10)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.225	1.225
Lower Limit		0.280	0.280
Upper Limit		5.883	5.833
Weeks to First Observed Tumor	96	97	101
Pituitary: Adenoma, NOS (b)	9/45(20)	7/48(15)	10/44(23)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.729	1.136
Lower Limit		0.252	0.460
Upper Limit		2.013	2.850
Weeks to First Observed Tumor	101	71	82
Pituitary: Adenoma, NOS or Carcinoma, NOS (b)	10/45(22)	8/48(17)	11/44(25)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.750	1.125
Lower Limit		0.283	0.484
Upper Limit		1.919	2.646
Weeks to First Observed Tumor	101	71	82

Table 7. Analyses of the Incidence of Primary Tumors in Male Rats Fed Diets Containing Gum Arabic (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Adrenal: Cortical Adenoma (b)	0/47(0)	1/50(2)	3/49(6)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		Infinite	Infinite
Lower Limit		0.050	0.578
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	99	105
Adrenal: Pheochromocytoma (b)	13/47(28)	11/50(22)	9/49(18)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.795	0.664
Lower Limit		0.360	0.278
Upper Limit		1.729	1.515
Weeks to First Observed Tumor	98	78	98
Adrenal: Pheochromocytoma or Pheochromocytoma, Malignant (b)	14/47(30)	11/50(22)	9/49(18)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.739	0.617
Lower Limit		0.339	0.262
Upper Limit		1.569	1.376
Weeks to First Observed Tumor	98	78	98

Table 7. Analyses of the Incidence of Primary Tumors in Male Rats Fed Diets Containing Gum Arabic (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Thyroid: C-Cell Adenoma (b)	3/47(6)	3/45(7)	4/48(8)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.044	1.306
Lower Limit		0.147	0.234
Upper Limit		7.414	8.482
Weeks to First Observed Tumor	92	105	105
Thyroid: C-Cell Carcinoma (b)	0/47(0)	3/45(7)	1/48(2)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		Infinite	Infinite
Lower Limit		0.630	0.053
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	97	103
Thyroid: C-Cell Adenoma or Carcinoma (b)	3/47(6)	6/45(13)	5/48(10)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		2.089	1.632
Lower Limit		0.477	0.338
Upper Limit		12.215	9.987
Weeks to First Observed Tumor	92	97	103

Table 7. Analyses of the Incidence of Primary Tumors in Male Rats Fed Diets Containing Gum Arabic (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Mammary Gland: Fibroadenoma (b)	1/50(2)	0/50(0)	3/50(6)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.000	3.000
Lower Limit		0.000	0.251
Upper Limit		18.658	154.270
Weeks to First Observed Tumor	105	--	103
Preputial Gland: Adenoma, NOS (b)	3/50(6)	1/50(2)	4/50(8)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.333	1.333
Lower Limit		0.006	0.238
Upper Limit		3.983	8.684
Weeks to First Observed Tumor	105	105	98
Preputial Gland: Adenoma, NOS or Carcinoma, NOS (b)	4/50(8)	2/50(4)	5/50(10)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.500	1.250
Lower Limit		0.047	0.286
Upper Limit		3.318	5.954
Weeks to First Observed Tumor	82	105	79

Table 7. Analyses of the Incidence of Primary Tumors in Male Rats Fed Diets Containing Gum Arabic (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Testis: Interstitial-Cell Tumor (b)	36/44(82)	45/50(90)	42/49(86)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.100	1.048
Lower Limit		0.917	0.864
Upper Limit		1.285	1.263
Weeks to First Observed Tumor	82	65	79

(a) Dosed groups received doses of 25,000 or 50,000 ppm in the diet.
(b) Number of tumor-bearing animals/number of animals examined at site (percent).
(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the untreated control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.
(d) A negative trend (N) indicates a lower incidence in a dosed group than in a control group.
(e) The 95 percent confidence interval of the relative risk between each dosed group and the control group.

Table 8. Analyses of the Incidence of Primary Tumors in Female Rats Fed Diets Containing Gum Arabic (a)

Topography: Morphology	Control	Low Dose	High Dose
Hematopoietic System:			
Leukemia (b)	10/50(20)	7/50(14)	9/50(18)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.700	0.900
Lower Limit		0.246	0.354
Upper Limit		1.869	2.249
Weeks to First Observed Tumor	73	94	83
Hematopoietic System:			
Malignant Lymphoma or Leukemia (b)	11/50(22)	8/50(16)	9/50(18)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.727	0.818
Lower Limit		0.278	0.329
Upper Limit		1.811	1.976
Weeks to First Observed Tumor	73	80	83
Liver: Neoplastic Nodule (b)	3/49(6)	3/49(6)	2/50(4)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.000	0.653
Lower Limit		0.140	0.057
Upper Limit		7.126	5.457
Weeks to First Observed Tumor	105	100	97

Table 8. Analyses of the Incidence of Primary Tumors in Female Rats Fed Diets Containing Gum Arabic (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Pituitary: Adenoma, (NOS) (b)	26/50(52)	25/44(57)	22/47(47)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.093	0.900
Lower Limit		0.725	0.576
Upper Limit		1.626	1.396
Weeks to First Observed Tumor	81	82	43
Pituitary: Adenoma, NOS or Carcinoma, NOS (b)	28/50(56)	26/44(59)	22/47(47)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.055	0.836
Lower Limit		0.718	0.544
Upper Limit		1.531	1.276
Weeks to First Observed Tumor	81	82	43
Adrenal: Pheochromocytoma (b)	2/48(4)	5/49(10)	1/50(2)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		2.449	0.480
Lower Limit		0.424	0.008
Upper Limit		24.745	8.916
Weeks to First Observed Tumor	105	94	105

Table 8. Analyses of the Incidence of Primary Tumors in Female Rats Fed Diets Containing Gum Arabic (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Thyroid: C-Cell Adenoma (b)	3/49(6)	2/47(4)	2/49(4)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.695	0.667
Lower Limit		0.060	0.058
Upper Limit		5.793	5.565
Weeks to First Observed Tumor	81	105	105
Thyroid: C-Cell Adenoma or Carcinoma (b)	4/49(8)	3/47(6)	2/49(4)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.782	0.500
Lower Limit		0.120	0.047
Upper Limit		4.372	3.315
Weeks to First Observed Tumor	81	105	105
Mammary Gland: Fibroadenoma (b)	14/50(28)	12/50(24)	15/50(30)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.857	1.071
Lower Limit		0.404	0.542
Upper Limit		1.790	2.131
Weeks to First Observed Tumor	96	95	81

Table 8. Analyses of the Incidence of Primary Tumors in Female Rats Fed Diets Containing Gum Arabic (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Clitoral Gland: Carcinoma, NOS (b)	1/50(2)	2/50(4)	3/50(6)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		2.000	3.000
Lower Limit		0.108	0.251
Upper Limit		115.621	154.270
Weeks to First Observed Tumor	105	98	105
Clitoral Gland: Adenoma, NOS or Carcinoma, NOS (b)	3/50(6)	3/50(6)	5/50(10)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.000	1.667
Lower Limit		0.140	0.344
Upper Limit		7.133	10.225
Weeks to First Observed Tumor	105	98	93
Uterus: Endometrial Stromal Polyp (b)	14/49(29)	10/49(20)	10/50(20)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.714	0.700
Lower Limit		0.315	0.309
Upper Limit		1.554	1.525
Weeks to First Observed Tumor	100	80	82

Table 8. Analyses of the Incidence of Primary Tumors in Female Rats Fed Diets Containing Gum Arabic (a)

(Continued)

(a) Dosed groups received doses of 25,000 or 50,000 ppm in the diet.
(b) Number of tumor-bearing animals/number of animals examined at site (percent).
(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the untreated control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.
(d) A negative trend (N) indicates a lower incidence in a dosed group than in a control group.
(e) The 95 percent confidence interval of the relative risk between each dosed group and the control group.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

Throughout most of the study, mean body weights of dosed and control mice were comparable (Figure 3 and Table 9). No compound-related clinical signs were observed. Mean daily feed consumption was 86% (6.3/7.3) for low-dose male mice, 85% (6.2/7.3) for high-dose male mice, and 88% (7.8/8.9) for low- and high-dose female mice, compared with controls (Appendix G).

B. Survival (Mice)

Estimates of the probabilities of survival of male and female mice fed diets containing gum arabic at the concentrations of this bioassay, together with those of the control group, are shown by the Kaplan and Meier curves in Figure 4. No significant differences in survival were observed between any of the groups of either sex of mice.

In male mice, 38/50 (76%) of the controls, 41/50 (82%) of the low-dose, and 40/50 (80%) of the high-dose group lived to the end of the study at 105 weeks. In female mice, 36/50 (72%) of the controls, 40/50 (80%) of the low-dose, and 39/50 (78%) of the high-dose group lived to the end of the study at 105 weeks.

C. Pathology (Mice)

Histopathologic findings on neoplasms in mice are summarized in Appendix B, Tables B1 and B2; findings on nonneoplastic lesions are summarized in Appendix D, Tables D1 and D2.

The most frequent neoplasms found in all groups were those of the hematopoietic system in both sexes and tumors of the lung and liver in males.

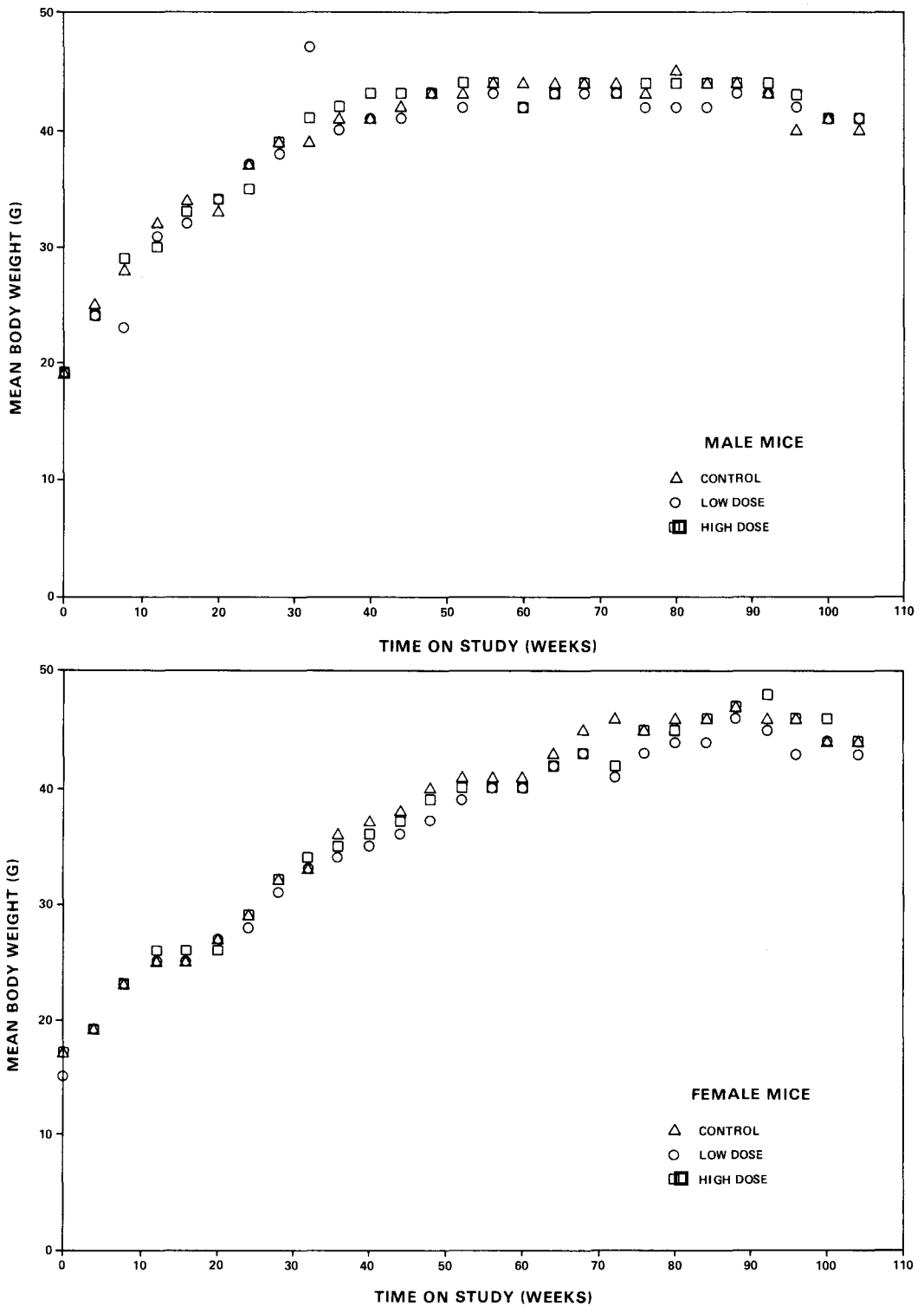

Figure 3. Growth Curves for Mice Fed Diets Containing Gum Arabic

Table 9. Mean Body Weight Change (Relative to Controls) of Mice Fed Diets Containing Gum Arabic

	Week No.	Mean Body Weight Change (grams) Control	Low Dose	High Dose	Weight Change Relative to Controls (a) (Percent) Low Dose	High Dose
	0	19(b)	19(b)	19(b)		
Male	4	6	5	5	-17	-17
Mice	24	18	18	16	0	-11
	44	23	22	24	-4	+4
	64	25	24	24	-4	-4
	84	25	23	25	-8	0
	104	21	22	22	+5	+5
	0	17(b)	15(b)	17(b)		
Female	4	2	4	2	+100	0
Mice	24	12	13	12	+8	0
	44	21	21	20	0	-5
	64	26	27	25	+4	-4
	84	29	29	29	0	0
	104	27	28	27	+4	0

(d) Weight Change Relative to Controls =

$$\frac{\text{Weight Change (Dosed Group)} - \text{Weight Change (Control Group)}}{\text{Weight Change (Control Group)}} \times 100$$

(b) Initial weight

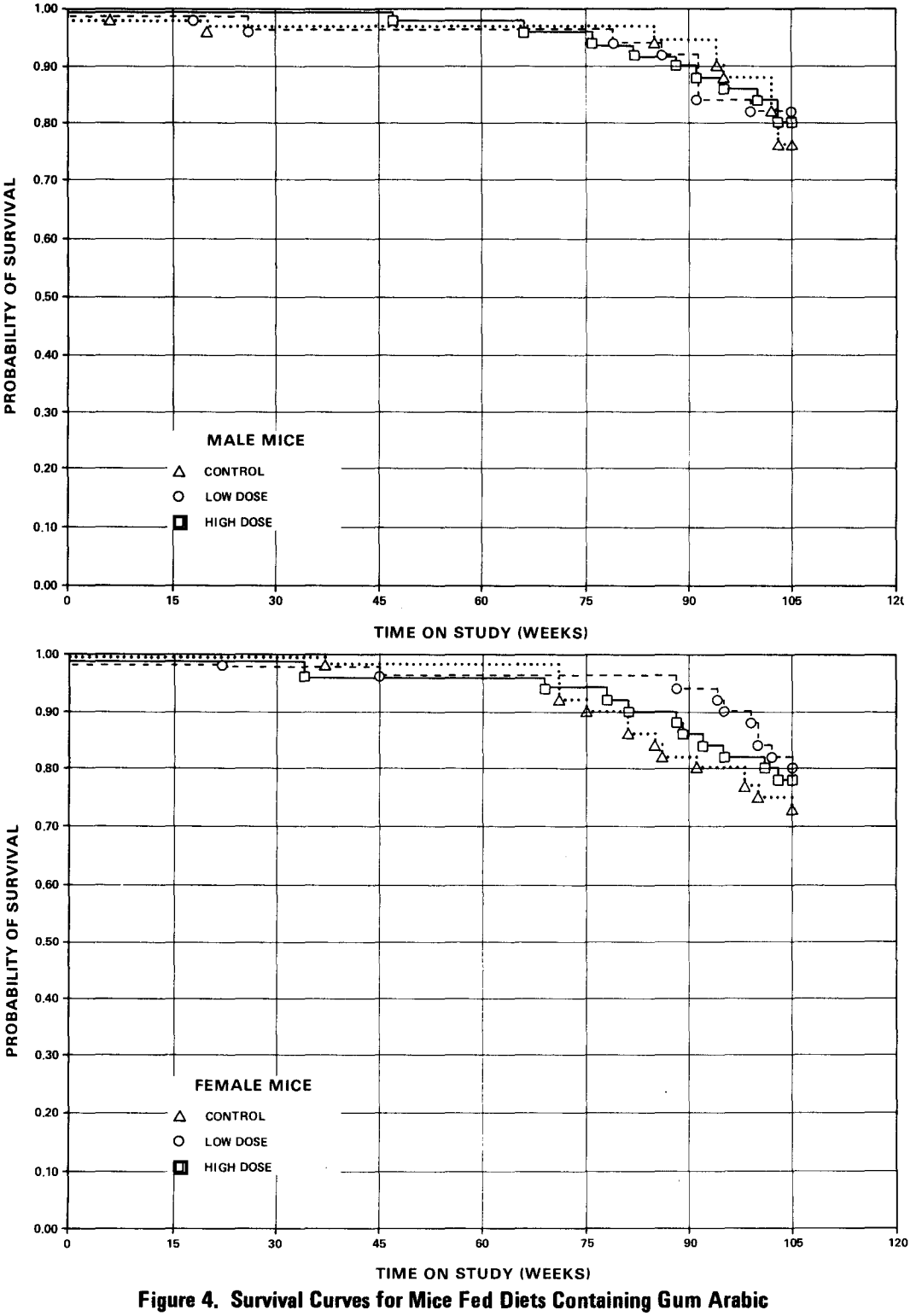

Figure 4. Survival Curves for Mice Fed Diets Containing Gum Arabic

Tumors of the liver were found in increased incidence in high-dose females (Table 10). Hepatocellular neoplasms were usually detected grossly as firm, nodular masses, often of a different color than normal liver. Microscopically, hepatocellular carcinomas were expansive masses of hepatocytes exhibiting loss of the normal architectural pattern. Both nuclei and cytoplasm varied from one region of the tumor to another. The tumor usually occupied more than half of the width of the liver. One tumor in a low-dose female had metastasized to the lung. Lesions classified as hepatocellular adenomas were smaller, better differentiated, and less pleomorphic.

A variety of nonneoplastic, inflammatory, and degenerative lesions occurred in all groups of mice. None could be related to administration of gum arabic.

The results of histopathologic examination showed, under the conditions of this bioassay, a marginal increase (although not statistically significant) in the number of hepatocellular adenomas, carcinomas, or neoplasms in high-dose female mice.

D. Statistical Analyses of Results (Mice)

Tables 11 and 12 contain the statistical analyses of those primary tumors that occurred in at least two animals of one group and with an incidence of at least 5% in one or more groups.

Hemangiomas of the circulatory system in male mice were observed in increased incidence in the high-dose group (0/49, 0% in the controls; 0/50, 0% in the low-dose; and 3/50, 6% in the high-dose group). The Cochran-Armitage test for linear trend was statistically significant in the positive direction (P=0.038). The Fisher exact tests were not significant. The historical records at this laboratory indicate an incidence of 15/852 (1.8%) male mice with hemangiomas. The incidence of mice with hemangiomas or hemangiosarcomas of the circulatory system was not significant in either sex. The incidence

Table 10. Incidences of Tumors of the Liver in Mice Fed Diets Containing Gum Arabic

	Males			Females		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
Liver						
(No. mice with tissues examined	49	49	50	49	50	50
Neoplasm, NOS	0	0	0	1	0	0
Hepatocellular adenoma	4	0	6	2	0	6
Hepatocellular carcinoma	13	11	10	1	2	6
No. of mice with either hepatocellular adenoma, carcinoma, or neoplasm, NOS	16(a)	11	15(a)	4	2	10(a)

(a) Some animals had both an hepatocellular adenoma and an hepatocellular carcinoma

of untreated male mice with hemangiomas or hemangiosarcomas of the circulatory system observed at this laboratory is 31/852 (3.6%). In female mice, this tumor was not observed in statistically significant proportions.

Hepatocellular adenomas, carcinomas, or neoplasms (unspecified) in female mice were observed in increased incidence in the high-dose group compared with the control group (4/49, 8% in the controls; 2/50, 4% in the low-dose; and 10/50, 20% in the high-dose group). The Cochran-Armitage test for linear trend was statistically significant in the positive direction (P=0.040), but the Fisher exact test between the high-dose group and the control group was not significant (P=0.080). By life table analysis, the dose-response trend was significant (P=0.044), but the high-dose effect was not. The historical records at this laboratory indicate the incidence of control female B6C3F1 mice with adenomas or carcinomas has been 77/859 (9.0%) with a range of 2% to 20.4%. Similarly, the trend in the incidence of hepatocellular carcinomas in female mice (1/49, 2/50, 6/50) was significant (P=0.031); comparing the control rate with the high-dose incidence was not significant (P=0.059). In male mice, this tumor was not observed in statistically significant proportions.

Neither time adjusted analysis, eliminating those animals dying before 52 weeks, nor life table analyses, using the week an animal died as the time point of examination for tumors, materially affected the previously reported results.

The conclusion based on statistical analysis is that there was no site at which an increase in tumor incidence could be clearly associated with the administration of the chemical.

Table 11. Analyses of the Incidence of Primary Tumors in Male Mice Fed Diets Containing Gum Arabic (a)

Topography: Morphology	Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma (b)	9/49(18)	5/49(10)	4/50(8)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.556	0.436
Lower Limit		0.157	0.104
Upper Limit		1.705	1.448
Weeks to First Observed Tumor	105	105	105
Lung: Alveolar/Bronchiolar Carcinoma (b)	4/49(8)	6/49(12)	9/50(18)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.500	2.205
Lower Limit		0.380	0.664
Upper Limit		6.811	9.203
Weeks to First Observed Tumor	102	105	105
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma (b)	12/49(24)	10/49(20)	12/50(24)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.883	0.980
Lower Limit		0.357	0.448
Upper Limit		1.901	2.147
Weeks to First Observed Tumor	102	105	105

Table 11. Analyses of the Incidence of Primary Tumors in Male Mice Fed Diets Containing Gum Arabic (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Hematopoietic System:			
Lymphoma, Malignant,			
Lymphocytic Type (b)	3/49(6)	4/50(8)	4/50(8)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.307	1.307
Lower Limit		0.233	0.233
Upper Limit		8.508	8.508
Weeks to First Observed Tumor	102	105	105
Hematopoietic System:			
Lymphoma, Malignant,			
Mixed Type (b)	4/49(8)	2/50(4)	1/50(2)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.490	0.245
Lower Limit		0.046	0.005
Upper Limit		3.251	2.362
Weeks to First Observed Tumor	105	105	105
Hematopoietic System:			
Malignant Lymphoma (b)	9/49(18)	6/50(12)	9/50(18)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.653	0.980
Lower Limit		0.207	0.377
Upper Limit		1.895	2.550
Weeks to First Observed Tumor	95	105	76

Table 11. Analyses of the Incidence of Primary Tumors in Male Mice Fed Diets Containing Gum Arabic (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Hematopoietic System:			
Malignant Lymphoma or Leukemia (b)	9/49(18)	7/50(14)	9/50(18)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.762	0.980
Lower Limit		0.262	0.377
Upper Limit		2.115	2.550
Weeks to First Observed Tumor	95	105	76
Circulatory System:			
Hemangioma (b)	0/49(0)	0/50(0)	3/50(6)
P Values (c),(d)	P=0.038	N.S.	N.S.
Relative Risk (Control) (e)		--	Infinite
Lower Limit		--	0.590
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	105
Circulatory System:			
Hemangiosarcoma (b)	2/49(4)	3/50(6)	2/50(4)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.470	0.980
Lower Limit		0.176	0.074
Upper Limit		16.980	13.058
Weeks to First Observed Tumor	105	105	95

Table 11. Analyses of the Incidence of Primary Tumors in Male Mice Fed Diets Containing Gum Arabic (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Circulatory System:			
Hemangioma or Hemangiosarcoma (b)	2/49(4)	3/50(6)	5/50(10)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.470	2.450
Lower Limit		0.176	0.424
Upper Limit		16.980	24.778
Weeks to First Observed Tumor	105	105	95
Liver: Hepatocellular Adenoma (b)	4/49(8)	0/49(0)	6/50(12)
P Values (c),(d)	N.S.	N.S.	N.S.
Departure from Linear Trend (f)	P=0.021		
Relative Risk (Control) (e)		0.000	1.470
Lower Limit		0.000	0.372
Upper Limit		1.078	6.681
Weeks to First Observed Tumor	94	--	82
Liver: Hepatocellular			
Carcinoma (b)	13/49(27)	11/49(22)	10/50(20)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.846	0.754
Lower Limit		0.375	0.328
Upper Limit		1.839	1.679
Weeks to First Observed Tumor	85	86	66

Table 11. Analyses of the Incidence of Primary Tumors in Male Mice Fed Diets Containing Gum Arabic (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Liver: Hepatocellular Adenoma or Carcinoma (b)	16/49(33)	11/49(22)	15/50(30)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.688	0.919
Lower Limit		0.323	0.479
Upper Limit		1.408	1.755
Weeks to First Observed Tumor	85	86	66
Adrenal: Cortical Adenoma (b)	3/45(7)	1/48(2)	1/47(2)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.313	0.319
Lower Limit		0.006	0.006
Upper Limit		3.725	3.801
Weeks to First Observed Tumor	105	105	105
Pituitary: Adenoma, NOS (b)	1/40(3)	0/36(0)	2/38(5)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.000	2.105
Lower Limit		0.000	0.114
Upper Limit		20.582	120.862
Weeks to First Observed Tumor	105	--	105

Table 11. Analyses of the Incidence of Primary Tumors in Male Mice Fed Diets Containing Gum Arabic (a)

(Continued)

(a) Dosed groups received doses of 25,000 or 50,000 ppm in the diet.
(b) Number of tumor-bearing animals/number of animals examined at site (percent).
(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the untreated control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.
(d) A negative trend (N) indicates a lower incidence in a dosed group than in a control group.
(e) The 95 percent confidence interval of the relative risk between each dosed group and the control group.
(f) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

Table 12. Analyses of the Incidence of Primary Tumors in Female Mice Fed Diets Containing Gum Arabic (a)

Topography: Morphology	Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma (b)	2/48(4)	5/49(10)	1/50(2)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		2.449	0.480
Lower Limit		0.424	0.008
Upper Limit		24.745	8.916
Weeks to First Observed Tumor	105	105	105
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma (b)	3/48(6)	7/49(14)	1/50(2)
P Values (c),(d)	N.S.	N.S.	N.S.
Departure from Linear Trend (f)	P=0.027		
Relative Risk (Control) (e)		2.286	0.320
Lower Limit		0.558	0.006
Upper Limit		13.001	3.822
Weeks to First Observed Tumor	98	95	105
Hematopoietic System: Lymphoma, Malignant, Lymphocytic Type (b)	8/49(16)	7/50(14)	5/50(10)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.858	0.613
Lower Limit		0.287	0.169
Upper Limit		2.497	1.969
Weeks to First Observed Tumor	105	105	105

Table 12. Analyses of the Incidence of Primary Tumors in Female Mice Fed Diets Containing Gum Arabic (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Hematopoietic System:			
Lymphoma, Malignant,			
Histiocytic Type (b)	1/49(2)	1/50(2)	3/50(6)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.980	2.940
Lower Limit		0.013	0.246
Upper Limit		75.404	151.180
Weeks to First Observed Tumor	86	88	105
Hematopoietic System:			
Lymphoma, Malignant,			
Mixed Type (b)	8/49(16)	5/50(10)	11/50(22)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.613	1.348
Lower Limit		0.169	0.542
Upper Limit		1.969	3.529
Weeks to First Observed Tumor	105	100	105
Hematopoietic System			
Lymphoma, Malignant, NOS (b)	1/49(2)	3/50(6)	2/50(4)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		2.940	1.960
Lower Limit		0.246	0.106
Upper Limit		151.180	113.312
Weeks to First Observed Tumor	81	99	101

Table 12. Analyses of the Incidence of Primary Tumors in Female Mice Fed Diets Containing Gum Arabic (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Hematopoietic System:			
Lymphoma (b)	18/49(37)	16/50(32)	21/50(42)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.871	1.143
Lower Limit		0.474	0.669
Upper Limit		1.590	1.972
Weeks to First Observed Tumor	81	88	101
Hematopoietic System:			
Lymphoma or Leukemia (b)	19/49(39)	16/50(32)	22/50(44)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.825	1.135
Lower Limit		0.454	0.679
Upper Limit		1.485	1.910
Weeks to First Observed Tumor	81	88	78
Liver: Hepatocellular Adenoma (b)	2/49(4)	0/50(0)	6/50(12)
P Values (c),(d)	N.S.	N.S.	N.S.
Departure from Linear Trend (f)	P=0.039		
Relative Risk (Control) (e)		0.000	2.940
Lower Limit		0.000	0.558
Upper Limit		3.313	28.662
Weeks to First Observed Tumor	98	--	105

Table 12. Analyses of the Incidence of Primary Tumors in Female Mice Fed Diets Containing Gum Arabic (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma (b)	1/49(2)	2/50(4)	6/50(12)
P Values (c),(d)	P=0.031	N.S.	N.S.
Relative Risk (Control) (e)		1.960	5.880
Lower Limit		0.106	0.753
Upper Limit		113.312	264.516
Weeks to First Observed Tumor	105	105	105
Liver: Hepatocellular Adenoma, Carcinoma, or Neoplasm, NOS (b)	4/49(8)	2/50(4)	10/50(20)
P Values (c),(d)	P=0.040	N.S.	N.S.
Relative Risk (Control) (e)		0.490	2.450
Lower Limit		0.046	0.764
Upper Limit		3.251	10.037
Weeks to First Observed Tumor	75	105	105
Uterus: Endometrial Stromal Polyp (b)	1/48(2)	1/49(2)	4/49(8)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.980	3.918
Lower Limit		0.013	0.407
Upper Limit		75.342	188.792
Weeks to First Observed Tumor	105	105	105

Table 12. Analyses of the Incidence of Primary Tumors in Female Mice Fed Diets Containing Gum Arabic (a)

(Continued)

(a) Dosed groups received doses of 25,000 or 50,000 ppm in the diet.
(b) Number of tumor-bearing animals/number of animals examined at site (percent).
(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the untreated control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.
(d) A negative trend (N) indicates a lower incidence in a dosed group than in a control group.
(e) The 95 percent confidence interval of the relative risk between each dosed group and the control group.
(f) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

V. DISCUSSION

Fifty Fischer 344 rats and B6C3F1 mice of each sex were fed diets containing 25,000 ppm or 50,000 ppm of gum arabic for 103 weeks to determine the potential carcinogenicity in these laboratory animals. The doses chosen represent the suggested maximum levels (5%) of a chemical to be added to feed (NCI, 1976). When the prechronic studies do not give data that are useful for selecting more definitive dose levels, the NTP currently adheres (most often) to this recommendation.

Mean body weights of dosed mice of either sex and of dosed male rats were comparable with those of the controls throughout the study. The mean body weights of dosed female rats were slightly lower than those of the controls. No compound-related clinical signs or effects on survival were observed.

Feed consumption in rats was 87% to 94% that of controls (males: 94.1% for low-dose and 87.8% for high-dose; females: 87.7% for low-dose and 87.2% for high-dose); values for mice were 85% to 88% (males:86.3% for low-dose and 84.9% for high-dose; females: 87.6% for low- and high-dose).

A statistically significant (P=0.040) increasing trend was observed for the incidence of liver tumors in female mice (4/49, 8%, controls; 2/50, 4%, low-dose; 10/50, 20%, high-dose); the high-dose incidence, when compared with controls, was not statistically different. These results could be considered as a marginal effect; yet, when viewed from an historic vantage point and using life table analysis, a conclusion other than not carcinogenic would be misleading. The usual types of tumors seen in aging F344 rats and B6C3F1 mice were observed in this study, but the incidences of these tumors were not considered compound related.

Besides gum arabic, four other "gums" have been tested recently by the NCI/NTP bioassay program; each was added to the diet (2.5% and 5.0%) and fed for 104 weeks to F344 rats and B6C3F1 mice of each sex. Under these test conditions, all were considered not carcinogenic (agar, NTP 1982a; guar gum, NTP 1982b; locust bean gum, NTP 1982c; and tara gum, NTP 1982d).

VI. CONCLUSION

Under the conditions of this bioassay, gum arabic was not carcinogenic for F344 rats or B6C3F1 mice of either sex.

VII. BIBLIOGRAPHY

Anderson, D. and Dea, I., Recent advances in the chemistry of Acacia gums. J. Soc. Cosmet. Chem. 22:61-73, 1971.

Armitage, P., Statistical Methods in Medical Research, John Wiley & Sons, Inc., New York, 1971, pp. 362-365.

Bailey, D. and Morgareidge, K., Comparative acute oral toxicity of 12 food grade gums in the mouse, rat, hamster, and rabbit. Food and Drug Research Labs Papers No. 124, 1976.

Berenblum, I., ed., Carcinogenicity Testing: A Report of the Panel on Carcinogenicity of the Cancer Research Commission of UICC, Vol. 2, International Union Against Cancer, Geneva, 1969.

CFR, U.S. Code of Federal Regulations 21:121.101, 1974.

Cox, D. R., Analysis of Binary Data, Methuen & Co., Ltd., London, 1970, pp. 48-52.

Cox, D. R. Regression models and life tables. J. R. Stat. Soc. B34:187-220, 1972.

Furia, T., ed., CRC Handbook of Food Additives, CRC Press, Cleveland, Ohio, 1972, pp. 295-359.

Gart, J. J., The comparison of proportions: a review of significance tests, confidence limits and adjustments for stratification. Rev. Int. Stat. Inst. 39:148-169, 1971.

Green, S., Present and future uses of mutagenicity tests for assessment of the safety of food additives. J. Environ. Pathol. Toxicol. 1:49-54, 1977.

Kaplan, E. L. and Meier, P., Nonparametric estimation from incomplete observations. J. Amer. Stat. Assoc. 53:457-481, 1958.

Kirk, R. E. and Othmer, D. F., eds. Encyclopedia of Chemical Technology, 2nd ed. Vol. 10, Interscience Publishers, 1966, pp. 744-745.

Life Sciences Research Office, Evaluation of the health aspects of gum arabic as a food ingredient, Federation of American Societies for Experimental Biology, Bethesda, Maryland, March 1973.

Linhart, M. S., Cooper, J. A., Martin, R. L., Page, N. P., and Peters, J. A., Carcinogenesis bioassay data system. Comp. Biomed. Res. 7:230-248, 1974.

McNulty, J. A., J. Assoc. Off. Anal. Chem. 43(3):624-632, 1960.

Miller, R. G., Jr., Simultaneous Statistical Inference, McGraw-Hill Book Co., New York, 1966, pp. 6-10.

NCI, National Cancer Institute, Guidelines for Carcinogen Bioassay in Small Rodents. DHEW Publication No. (NIH) 76-801, Carcinogenesis Testing Program, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, 1976.

NTP, National Toxicology Program, NCI/NTP Technical Report on the Carcinogenesis Bioassay of Agar, NTP TR 230, Department of Health and Human Services, Research Triangle Park, North Carolina, 1982a.

NTP, National Toxicology Program, NCI/NTP Technical Report on the Carcinogenesis Bioassay of Guar Gum, NTP TR 227, Department of Health and Human Services, Research Triangle Park, North Carolina, 1982b.

NTP, National Toxicology Program, NCI/NTP Technical Report on the Carcinogenesis Bioassay of Locust Bean Gum, NTP TR 221, Department of Health and Human Services, Research Triangle Park, North Carolina, 1982c.

NTP, National Toxicology Program, NCI/NTP Technical Report on the Carcinogenesis Bioassay of Tara Gum, NTP TR 224, Department of Health and Human Services, Research Triangle Park, North Carolina, 1982d.

Saffiotti, U., Montesano, R., Sellakumar, A. R., Cefis, F., and Kaufman, D. G., Respiratory tract carcinogenesis in hamsters induced by different numbers of administrations of benzo(a)pyrene and ferric oxide. Cancer Res. 32:1073-1081, 1972.

Tarone, R. E., Tests for trend in life table analysis. Biometrika 62:679-682, 1975.

USP, The Pharmacopeia of the United States of America, 18th ed., Mack Printing Company, Easton, Pennsylvania, 1970, pp. 378-379.

Varma, R., Varma, R. S., and Wardl, A. H., J. Chromatog. 77:22, 1973.

Ward, J. M., Goodman, D. G., Griesemer, R. A., Hardisty, J. F., Schueler, R. L., Squire, R. A., and Strandberg, J. D., Quality assurance for pathology in rodent carcinogenesis tests. J. Environ. Path. Toxicol. 2:371-378, 1978.

APPENDIX A

Summary of the Incidence of Neoplasms in Rats Fed Diets Containing Gum Arabic

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS FED DIETS CONTAINING GUM ARABIC

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
PAPILLOMA, NOS			2 (4%)
SQUAMOUS CELL PAPILLOMA	1 (2%)	1 (2%)	
BASAL-CELL CARCINOMA		1 (2%)	
*SUBCUT TISSUE	(50)	(50)	(50)
SARCOMA, NOS		1 (2%)	
FIBROMA	2 (4%)	2 (4%)	1 (2%)
NEURILEMOMA	1 (2%)		
RESPIRATORY SYSTEM			
#LUNG	(50)	(50)	(50)
CARCINOMA, NOS, METASTATIC	1 (2%)		
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (2%)	
PAPILLARY ADENOCARCINOMA, METAST			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	6 (12%)	1 (2%)	
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (2%)	3 (6%)	1 (2%)
LEUKEMIA,NOS	10.(20%)	15 (30%)	14 (28%)
LYMPHOCYTIC LEUKEMIA			1 (2%)
#LIVER	(49)	(50)	(50)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (2%)		
CIRCULATORY SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(48)	(49)	(49)
SARCOMA, NOS		2 (4%)	1 (2%)
FIBROSARCOMA		1 (2%)	
#LIVER	(49)	(50)	(50)
NEOPLASTIC NODULE	3 (6%)	2 (4%)	4 (8%)
HEPATOCELLULAR CARCINOMA	1 (2%)	3 (6%)	1 (2%)
#STOMACH	(46)	(49)	(48)
SQUAMOUS CELL PAPILLOMA	1 (2%)		
LEIOMYOSARCOMA			1 (2%)
#CECUM	(42)	(48)	(46)
ADENOCARCINOMA, NOS			1 (2%)
URINARY SYSTEM			
#KIDNEY	(48)	(50)	(50)
SARCOMA, NOS		1 (2%)	
#KIDNEY/PELVIS	(48)	(50)	(50)
TRANSITIONAL-CELL PAPILLOMA	1 (2%)		
ENDOCRINE SYSTEM			
#PITUITARY	(45)	(48)	(44)
CARCINOMA,NOS	1 (2%)	1 (2%)	1 (2%)
ADENOMA, NOS	9 (20%)	7 (15%)	10 (23%)
CHROMOPHOBE ADENOMA	1 (2%)	2 (4%)	1 (2%)
#ADRENAL	(47)	(50)	(49)
CORTICAL ADENOMA		1 (2%)	3 (6%)
PHEOCHROMOCYTOMA	13 (28%)	11 (22%)	9 (18%)
PHEOCHROMOCYTOMA, MALIGNANT	1 (2%)		
#THYROID	(47)	(45)	(48)
C-CELL ADENOMA	3 (6%)	3 (7%)	4 (8%)
C-CELL CARCINOMA		3 (7%)	1 (2%)
#THYROID FOLLICLE	(47)	(45)	(48)
PAPILLARY ADENOCARCINOMA	1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#PANCREATIC ISLETS	(41)	(48)	(48)
ISLET-CELL ADENOMA		1 (2%)	1 (2%)
ISLET-CELL CARCINOMA		1 (2%)	1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
ADENOCARCINOMA, NOS	1 (2%)		
PAPILLARY ADENOCARCINOMA			1 (2%)
FIBROADENOMA	1 (2%)		3 (6%)
*PREPUTIAL GLAND	(50)	(50)	(50)
CARCINOMA,NOS	1 (2%)	1 (2%)	1 (2%)
SQUAMOUS CELL CARCINOMA			1 (2%)
ADENOMA, NOS	3 (6%)	1 (2%)	4 (8%)
#PROSTATE	(40)	(45)	(44)
ADENOMA, NOS	1 (3%)	1 (2%)	1 (2%)
#TESTIS	(44)	(50)	(49)
INTERSTITIAL-CELL TUMOR	36 (82%)	45 (90%)	42 (86%)
NERVOUS SYSTEM			
#BRAIN	(49)	(49)	(50)
ASTROCYTOMA		1 (2%)	
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(50)	(50)	(50)
ADENOMA, NOS	1 (2%)		
*ZYMBAL'S GLAND	(50)	(50)	(50)
CERUMINOUS CARCINOMA		1 (2%)	
MUSCULOSKELETAL SYSTEM			
*SKULL	(50)	(50)	(50)
OSTEOSARCOMA		1 (2%)	
*FEMUR	(50)	(50)	(50)
OSTEOSARCOMA		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*PERITONEUM	(50)	(50)	(50)
MESOTHELIOMA, NOS		1 (2%)	
*MESENTERY	(50)	(50)	(50)
LIPOMA	1 (2%)		
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(50)
NEOPLASM, NOS			1 (2%)
SARCOMA, NOS, METASTATIC		1 (2%)	
OSTEOSARCOMA, METASTATIC		1 (2%)	
NECK			
SARCOMA, NOS, INVASIVE			1
SITE UNKNOWN			
CARCINOMA,NOS	1		
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	18	15	13
MORIBUND SACRIFICE	6	9	8
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	26	26	29
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	48	49	48
TOTAL PRIMARY TUMORS	103	117	112
TOTAL ANIMALS WITH BENIGN TUMORS	42	47	45
TOTAL BENIGN TUMORS	75	76	81
TOTAL ANIMALS WITH MALIGNANT TUMORS	25	29	25
TOTAL MALIGNANT TUMORS	25	38	26
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	2	2
TOTAL SECONDARY TUMORS	1	2	2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	3	3	5
TOTAL UNCERTAIN TUMORS	3	3	5
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS FED DIETS CONTAINING GUM ARABIC

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
SQUAMOUS CELL PAPILLOMA		2 (4%)	
SQUAMOUS CELL CARCINOMA			1 (2%)
KERATOACANTHOMA			1 (2%)
*SUBCUT TISSUE	(50)	(50)	(50)
SARCOMA, NOS			1 (2%)
FIBROSARCOMA			1 (2%)
LIPOMA			1 (2%)
CARCINOSARCOMA			1 (2%)
NEURILEMOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(50)	(49)	(50)
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (2%)	1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	1 (2%)	1 (2%)	
LEUKEMIA,NOS	10 (20%)	7 (14%)	9 (18%)
CIRCULATORY SYSTEM			
#UTERUS	(49)	(49)	(50)
HEMANGIOMA		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(49)	(49)	(50)
NEOPLASTIC NODULE	3 (6%)	3 (6%)	2 (4%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(50)	(44)	(47)
CARCINOMA,NOS	2 (4%)	1 (2%)	
ADENOMA, NOS	26 (52%)	25 (57%)	22 (47%)
CHROMOPHOBE ADENOMA	1 (2%)	1 (2%)	2 (4%)
#ADRENAL	(48)	(49)	(50)
CORTICAL ADENOMA		2 (4%)	1 (2%)
PHEOCHROMOCYTOMA	2 (4%)	5 (10%)	1 (2%)
#THYROID	(49)	(47)	(49)
FOLLICULAR-CELL CARCINOMA	1 (2%)		
C-CELL ADENOMA	3 (6%)	2 (4%)	2 (4%)
C-CELL CARCINOMA	1 (2%)	1 (2%)	
#THYROID FOLLICLE	(49)	(47)	(49)
PAPILLARY CYSTADENOMA, NOS	1 (2%)	1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
FIBROADENOMA	14 (28%)	12 (24%)	15 (30%)
*CLITORAL GLAND	(50)	(50)	(50)
CARCINOMA,NOS	1 (2%)	2 (4%)	3 (6%)
ADENOMA, NOS	2 (4%)	1 (2%)	2 (4%)
#UTERUS	(49)	(49)	(50)
SARCOMA, NOS	1 (2%)		1 (2%)
ENDOMETRIAL STROMAL POLYP	14 (29%)	10 (20%)	10 (20%)
ENDOMETRIAL STROMAL SARCOMA	1 (2%)	1 (2%)	
#CERVIX UTERI	(49)	(49)	(50)
LEIOMYOSARCOMA	1 (2%)		
#OVARY	(48)	(48)	(50)
GRANULOSA-CELL TUMOR			2 (4%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
NERVOUS SYSTEM			
#BRAIN	(50)	(50)	(49)
GRANULAR-CELL TUMOR, NOS		1 (2%)	
GLIOMA, NOS			1 (2%)
ASTROCYTOMA			1 (2%)
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(50)	(50)	(50)
ADENOMA, NOS		1 (2%)	
*ZYMBAL'S GLAND	(50)	(50)	(50)
CERUMINOUS CARCINOMA		1 (2%)	1 (2%)
MUSCULOSKELETAL SYSTEM			
*SKULL	(50)	(50)	(50)
OSTEOSARCOMA			1 (2%)
*VERTEBRA	(50)	(50)	(50)
CHORDOMA		1 (2%)	
BODY CAVITIES			
*ABDOMINAL WALL	(50)	(50)	(50)
LIPOMA		1 (2%)	
*MESENTERY	(50)	(50)	(50)
LIPOMA	1 (2%)		
ALL OTHER SYSTEMS			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATHa	10	6	9
MORIBUND SACRIFICE	6	8	9
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	34	36	32
ANIMAL MISSING			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	45	46	47
TOTAL PRIMARY TUMORS	86	85	83
TOTAL ANIMALS WITH BENIGN TUMORS	37	42	38
TOTAL BENIGN TUMORS	64	65	57
TOTAL ANIMALS WITH MALIGNANT TUMORS	18	16	20
TOTAL MALIGNANT TUMORS	19	16	22
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT	3	4	4
TOTAL UNCERTAIN TUMORS	3	4	4
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

a INCLUDES AUTOLYZED ANIMALS

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX B

Summary of the Incidence of Neoplasms in Mice Fed Diets Containing Gum Arabic

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE FED DIETS CONTAINING GUM ARABIC

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	49	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	49	50	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(49)	(50)	(50)
SARCOMA, NOS	1 (2%)		2 (4%)
FIBROMA		1 (2%)	
FIBROSARCOMA	2 (4%)	2 (4%)	
FIBROUS HISTIOCYTOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(49)	(49)	(50)
HEPATOCELLULAR CARCINOMA, METAST	2 (4%)	1 (2%)	2 (4%)
ALVEOLAR/BRONCHIOLAR ADENOMA	9 (18%)	5 (10%)	4 (8%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	4 (8%)	6 (12%)	9 (18%)
SARCOMA, NOS, METASTATIC			2 (4%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(49)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	1 (2%)		2 (4%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	2 (4%)	1 (2%)	2 (4%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)		1 (2%)
MALIGNANT LYMPHOMA, MIXED TYPE	4 (8%)	1 (2%)	
LEUKEMIA,NOS		1 (2%)	
*HEMATOPOIETIC SYSTEM	(49)	(50)	(50)
NEOPLASM, NOS		1 (2%)	
#SPLEEN	(47)	(48)	(49)
NEOPLASM, NOS			1 (2%)
MALIGNANT LYMPHOMA, MIXED TYPE			1 (2%)
#MEDIASTINAL L.NODE	(42)	(45)	(48)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#MESENTERIC L. NODE	(42)	(45)	(48)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
MALIGNANT LYMPHOMA, MIXED TYPE		1 (2%)	
#DUODENUM	(44)	(45)	(47)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (2%)	1 (2%)	
#JEJUNUM	(44)	(45)	(47)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	1 (2%)
#KIDNEY	(48)	(50)	(50)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
CIRCULATORY SYSTEM			
*MULTIPLE ORGANS	(49)	(50)	(50)
HEMANGIOSARCOMA	1 (2%)		
#SPLEEN	(47)	(48)	(49)
HEMANGIOMA			1 (2%)
HEMANGIOSARCOMA		2 (4%)	2 (4%)
#MYOCARDIUM	(49)	(48)	(50)
SARCOMA, NOS			1 (2%)
#LIVER	(49)	(49)	(50)
HEMANGIOMA			2 (4%)
HEMANGIOSARCOMA	1 (2%)	1 (2%)	2 (4%)
DIGESTIVE SYSTEM			
#LIVER	(49)	(49)	(50)
HEPATOCELLULAR ADENOMA	4 (8%)		6 (12%)
HEPATOCELLULAR CARCINOMA	13 (27%)	11 (22%)	10 (20%)
LIPOMA			1 (2%)
#STOMACH	(46)	(47)	(48)
ADENOCARCINOMA, NOS			1 (2%)
#JEJUNUM	(44)	(45)	(47)
ADENOCARCINOMA, NOS		1 (2%)	
*RECTUM	(49)	(50)	(50)
ADENOCARCINOMA, NOS			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(40)	(36)	(38)
ADENOMA, NOS	1 (3%)		2 (5%)
#ADRENAL	(45)	(48)	(47)
CORTICAL ADENOMA	3 (7%)	1 (2%)	1 (2%)
PHEOCHROMOCYTOMA	2 (4%)		
#THYROID	(45)	(46)	(49)
FOLLICULAR-CELL ADENOMA	1 (2%)	1 (2%)	
C-CELL TUMOR	1 (2%)		
C-CELL CARCINOMA			1 (2%)
#THYROID FOLLICLE	(45)	(46)	(49)
CYSTADENOMA, NOS	1 (2%)		
#PANCREATIC ISLETS	(45)	(46)	(49)
ISLET-CELL ADENOMA			1 (2%)
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(49)	(50)	(50)
ADENOMA, NOS	1 (2%)		
#TESTIS	(47)	(49)	(49)
INTERSTITIAL-CELL TUMOR		1 (2%)	1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(49)	(50)	(50)
ADENOMA, NOS	2 (4%)		1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
MUSCULOSKELETAL SYSTEM			
*VERTEBRAL COLUMN	(49)	(50)	(50)
OSTEOSARCOMA			1 (2%)
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(49)	(50)	(50)
HEPATOCELLULAR CARCINOMA, METAST	1 (2%)		
TAIL			
SARCOMA, NOS			1
FIBROSARCOMA		1	
OSTEOSARCOMA	1		
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	12	8	9
MORIBUND SACRIFICE		1	1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	38	41	40
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	36	28	40
TOTAL PRIMARY TUMORS	57	41	62
TOTAL ANIMALS WITH BENIGN TUMORS	19	8	16
TOTAL BENIGN TUMORS	24	9	21
TOTAL ANIMALS WITH MALIGNANT TUMORS	27	23	34
TOTAL MALIGNANT TUMORS	32	31	40
TOTAL ANIMALS WITH SECONDARY TUMORS#	3	1	4
TOTAL SECONDARY TUMORS	3	1	4
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	1	1	1
TOTAL UNCERTAIN TUMORS	1	1	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE B2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE FED DIETS CONTAINING GUM ARABIC

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING	1		
ANIMALS NECROPSIED	49	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	49	50	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(49)	(50)	(50)
FIBROSARCOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(48)	(49)	(50)
CARCINOMA, NOS, METASTATIC		1 (2%)	
HEPATOCELLULAR CARCINOMA, METAST		1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (4%)	5 (10%)	1 (2%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (2%)	2 (4%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(49)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	1 (2%)	2 (4%)	2 (4%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	6 (12%)	5 (10%)	2 (4%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)	1 (2%)	2 (4%)
MALIGNANT LYMPHOMA, MIXED TYPE	6 (12%)	3 (6%)	9 (18%)
LEUKEMIA,NOS	1 (2%)		1 (2%)
#SPLEEN	(47)	(48)	(49)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			2 (4%)
MALIGNANT LYMPHOMA, MIXED TYPE		1 (2%)	
#LYMPH NODE	(45)	(46)	(41)
ALVEOLAR/BRONCHIOLAR CA, METASTA	1 (2%)		
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (2%)		
#MANDIBULAR L. NODE	(45)	(46)	(41)
SARCOMA, NOS, METASTATIC		1 (2%)	
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#MESENTERIC L. NODE	(45)	(46)	(41)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (2%)		1 (2%)
#LIVER	(49)	(50)	(50)
MALIGNANT LYMPHOMA, MIXED TYPE	1 (2%)		
#DUODENUM	(45)	(48)	(48)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
#JEJUNUM	(45)	(48)	(48)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	
MALIGNANT LYMPHOMA, MIXED TYPE			1 (2%)
#ILEUM	(45)	(48)	(48)
MALIGNANT LYMPHOMA, MIXED TYPE			1 (2%)
#KIDNEY	(49)	(48)	(48)
MALIGNANT LYMPHOMA, MIXED TYPE	1 (2%)	1 (2%)	
CIRCULATORY SYSTEM			
#SPLEEN	(47)	(48)	(49)
HEMANGIOMA	1 (2%)	1 (2%)	
#HEART	(49)	(47)	(50)
ALVEOLAR/BRONCHIOLAR CA, METASTA	1 (2%)		
*VULVA	(49)	(50)	(50)
HEMANGIOMA			1 (2%)
#OVARY	(40)	(40)	(43)
HEMANGIOMA			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(49)	(50)	(50)
NEOPLASM, NOS	1 (2%)		
HEPATOCELLULAR ADENOMA	2 (4%)		6 (12%)
HEPATOCELLULAR CARCINOMA	1 (2%)	2 (4%)	6 (12%)
#STOMACH	(45)	(46)	(49)
SQUAMOUS CELL PAPILLOMA	1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(39)	(41)	(40)
CARCINOMA,NOS		1 (2%)	1 (3%)
ADENOMA, NOS	1 (3%)	1 (2%)	1 (3%)
ACIDOPHIL ADENOMA			1 (3%)
#ADRENAL	(48)	(44)	(43)
PHEOCHROMOCYTOMA		1 (2%)	1 (2%)
#THYROID	(45)	(45)	(43)
FOLLICULAR-CELL ADENOMA		1 (2%)	
FOLLICULAR-CELL CARCINOMA		1 (2%)	
C-CELL ADENOMA	1 (2%)		
#THYROID FOLLICLE	(45)	(45)	(43)
CYSTADENOMA, NOS			1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(49)	(50)	(50)
ACINAR-CELL CARCINOMA	1 (2%)	1 (2%)	
MIXED TUMOR, MALIGNANT	1 (2%)		
#UTERUS	(48)	(49)	(49)
SARCOMA, NOS	1 (2%)	1 (2%)	
ENDOMETRIAL STROMAL POLYP	1 (2%)	1 (2%)	4 (8%)
ENDOMETRIAL STROMAL SARCOMA		1 (2%)	
#OVARY	(40)	(40)	(43)
SERTOLI-CELL TUMOR			1 (2%)
TERATOMA, NOS	1 (3%)	1 (3%)	
NERVOUS SYSTEM			
#BRAIN	(49)	(49)	(50)
CARCINOMA, NOS, INVASIVE		1 (2%)	1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(49)	(50)	(50)
CARCINOMA,NOS		1 (2%)	
ADENOMA, NOS	2 (4%)	1 (2%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(49)	(50)	(50)
MESOTHELIOMA, NOS		1 (2%)	
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	13	10	10
MORIBUND SACRIFICE			1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	36	40	39
ANIMAL MISSING	1		

@ INCLUDES AUTOLYZED ANIMALS

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	30	33	31
TOTAL PRIMARY TUMORS	37	39	48
TOTAL ANIMALS WITH BENIGN TUMORS	10	11	16
TOTAL BENIGN TUMORS	11	11	18
TOTAL ANIMALS WITH MALIGNANT TUMORS	24	23	27
TOTAL MALIGNANT TUMORS	24	26	30
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	4	1
TOTAL SECONDARY TUMORS	2	4	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	2	2	
TOTAL UNCERTAIN TUMORS	2	2	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX C

Summary of the Incidence of Nonneoplastic Lesions in Rats Fed Diets Containing Gum Arabic

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS FED DIETS CONTAINING GUM ARABIC

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
EPIDERMAL INCLUSION CYST	1 (2%)		
EDEMA, NOS		1 (2%)	
HEMORRHAGIC CYST	1 (2%)		
ABSCESS, NOS			1 (2%)
RESPIRATORY SYSTEM			
*NOSE	(50)	(50)	(50)
SKIN TAG			1 (2%)
#LUNG	(50)	(50)	(50)
HEMORRHAGE	1 (2%)		1 (2%)
PNEUMONIA, CHRONIC MURINE		1 (2%)	
CALCIFICATION, FOCAL		1 (2%)	
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(48)	(50)	(49)
FIBROSIS	1 (2%)		
FIBROSIS, FOCAL	1 (2%)	1 (2%)	
HYPOPLASIA, NOS		1 (2%)	
HYPERPLASIA, NOS	2 (4%)	7 (14%)	2 (4%)
#SPLEEN	(46)	(50)	(50)
HEMATOMA, NOS			1 (2%)
ABSCESS, NOS			1 (2%)
FIBROSIS, FOCAL	1 (2%)		
HEMOSIDEROSIS			1 (2%)
HEMATOPOIESIS	6 (13%)	1 (2%)	2 (4%)
#MEDIASTINAL L.NODE	(47)	(47)	(48)
CONGESTION, NOS	1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#PANCREATIC L.NODE	(47)	(47)	(48)
CONGESTION, NOS			1 (2%)
#MESENTERIC L. NODE	(47)	(47)	(48)
CONGESTION, NOS			1 (2%)
CIRCULATORY SYSTEM			
#MESENTERIC L. NODE	(47)	(47)	(48)
LYMPHANGIECTASIS	3 (6%)	1 (2%)	1 (2%)
#HEART	(50)	(50)	(50)
THROMBOSIS, NOS		1 (2%)	1 (2%)
THROMBUS, MURAL	1 (2%)	2 (4%)	
#MYOCARDIUM	(50)	(50)	(50)
DEGENERATION, NOS	23 (46%)	27 (54%)	18 (36%)
#PANCREAS	(41)	(48)	(48)
PERIARTERITIS		1 (2%)	
#STOMACH	(46)	(49)	(48)
PERIARTERITIS		1 (2%)	
#TESTIS	(44)	(50)	(49)
PERIVASCULITIS		1 (2%)	
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(48)	(49)	(49)
INFLAMMATION, CHRONIC	2 (4%)		
#LIVER	(49)	(50)	(50)
CONGESTION, CHRONIC PASSIVE			1 (2%)
FIBROSIS		2 (4%)	
NECROSIS, FOCAL			1 (2%)
METAMORPHOSIS FATTY	7 (14%)	4 (8%)	4 (8%)
CYTOPLASMIC CHANGE, NOS	1 (2%)		
BASOPHILIC CYTO CHANGE	3 (6%)		1 (2%)
CLEAR-CELL CHANGE	2 (4%)	1 (2%)	
ANGIECTASIS		1 (2%)	
#LIVER/CENTRILOBULAR	(49)	(50)	(50)
NECROSIS, NOS		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#BILE DUCT	(49)	(50)	(50)
CYST, NOS			1 (2%)
HYPERPLASIA, NOS	33 (67%)	24 (48%)	26 (52%)
#STOMACH	(46)	(49)	(48)
MINERALIZATION			1 (2%)
INFLAMMATION, NOS		1 (2%)	
ULCER, NOS	5 (11%)	4 (8%)	2 (4%)
HYPERPLASIA, BASAL CELL	1 (2%)	3 (6%)	1 (2%)
ACANTHOSIS	1 (2%)	1 (2%)	
#GASTRIC MUCOSA	(46)	(49)	(48)
CALCIFICATION, NOS	4 (9%)		
#GASTRIC SUBMUCOSA	(46)	(49)	(48)
INFLAMMATION, NOS	1 (2%)		1 (2%)
INFLAMMATION, FOCAL		1 (2%)	
FIBROSIS			1 (2%)
#FORESTOMACH	(46)	(49)	(48)
HYPERPLASIA, BASAL CELL		1 (2%)	
#COLON	(42)	(48)	(46)
PARASITISM	5 (12%)	7 (15%)	7 (15%)
URINARY SYSTEM			
#KIDNEY	(48)	(50)	(50)
MINERALIZATION	2 (4%)		
HYDRONEPHROSIS			1 (2%)
CYST, NOS			1 (2%)
PARASITISM			1 (2%)
NEPHROPATHY	1 (2%)	1 (2%)	
NEPHROSIS, NOS	41 (85%)	43 (86%)	34 (68%)
NEPHROSIS, CHOLEMIC		3 (6%)	3 (6%)
CALCIFICATION, FOCAL	2 (4%)	1 (2%)	
#KIDNEY/PELVIS	(48)	(50)	(50)
HYPERPLASIA, EPITHELIAL	1 (2%)		
#URINARY BLADDER	(43)	(50)	(46)
CALCULUS, NOS	1 (2%)	1 (2%)	1 (2%)
HYPERPLASIA, EPITHELIAL		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#PITUITARY	(45)	(48)	(44)
CYST, NOS		1 (2%)	
HEMORRHAGE			1 (2%)
HEMORRHAGIC CYST	2 (4%)		
HYPERPLASIA, FOCAL	2 (4%)	1 (2%)	3 (7%)
VASCULARIZATION		2 (4%)	
#ADRENAL	(47)	(50)	(49)
HEMORRHAGE			1 (2%)
#ADRENAL CORTEX	(47)	(50)	(49)
HYPERPLASIA, NODULAR	3 (6%)	3 (6%)	
#ADRENAL MEDULLA	(47)	(50)	(49)
HYPERPLASIA, FOCAL	1 (2%)	6 (12%)	1 (2%)
#THYROID	(47)	(45)	(48)
HYPERPLASIA, C-CELL	1 (2%)	2 (4%)	1 (2%)
#PANCREATIC ISLETS	(41)	(48)	(48)
HYPERPLASIA, NOS	1 (2%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
GALACTOCELE		1 (2%)	1 (2%)
LACTATION		1 (2%)	
*PREPUTIAL GLAND	(50)	(50)	(50)
NECROSIS, NOS			1 (2%)
#PROSTATE	(40)	(45)	(44)
INFLAMMATION, ACUTE		1 (2%)	
INFLAMMATION ACUTE AND CHRONIC	4 (10%)	1 (2%)	
INFLAMMATION, CHRONIC	2 (5%)	1 (2%)	
ATROPHY, NOS			3 (7%)
HYPERPLASIA, NOS	2 (5%)		
HYPERPLASIA, EPITHELIAL		2 (4%)	2 (5%)
*SEMINAL VESICLE	(50)	(50)	(50)
INFLAMMATION ACUTE AND CHRONIC			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ATROPHY, NOS			3 (6%)
HYPERPLASIA, NOS	1 (2%)		
#TESTIS	(44)	(50)	(49)
HEMORRHAGE		1 (2%)	
INFARCT, NOS		1 (2%)	
CALCIFICATION, NOS		1 (2%)	
ATROPHY, NOS	2 (5%)	1 (2%)	3 (6%)
SPERMATOGENIC ARREST			1 (2%)
#TESTIS/TUBULE	(44)	(50)	(49)
ATROPHY, FOCAL		1 (2%)	
NERVOUS SYSTEM			
#BRAIN	(49)	(49)	(50)
HYDROCEPHALUS, NOS			1 (2%)
HEMORRHAGE		2 (4%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(50)	(50)	(50)
ABSCESS, NOS			1 (2%)
*MESENTERY	(50)	(50)	(50)
NECROSIS, FAT	4 (8%)	2 (4%)	1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(50)
CONGESTION, NOS		2 (4%)	
OMENTUM			
NECROSIS, FAT		1	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
SPECIAL MORPHOLOGY SUMMARY			
AUTO/NECROPSY/HISTO PERF	2		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS FED DIETS CONTAINING GUM ARABIC

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
ABSCESS, NOS	1 (2%)		
SKIN TAG	1 (2%)		
RESPIRATORY SYSTEM			
*NASAL TURBINATE	(50)	(50)	(50)
CONGESTION, NOS		1 (2%)	
#LUNG/BRONCHIOLE	(50)	(49)	(50)
METAPLASIA, NOS			1 (2%)
#LUNG	(50)	(49)	(50)
BRONCHOPNEUMONIA, NOS			1 (2%)
BRONCHOPNEUMONIA NECROTIZING			1 (2%)
PNEUMONIA, CHRONIC MURINE			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
HEMATOPOIESIS			1 (2%)
#BONE MARROW	(49)	(49)	(47)
FIBROSIS, FOCAL			1 (2%)
HYPERPLASIA, NOS			2 (4%)
#SPLEEN	(48)	(49)	(50)
HEMOSIDEROSIS		2 (4%)	
HEMATOPOIESIS	3 (6%)	7 (14%)	2 (4%)
#LYMPH NODE	(50)	(46)	(48)
CONGESTION, NOS			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, NOS	1 (2%)		
#ABDOMINAL LYMPH NODE	(50)	(46)	(48)
CONGESTION, NOS			1 (2%)
#MESENTERIC L. NODE	(50)	(46)	(48)
CONGESTION, NOS			1 (2%)
#THYMUS	(37)	(43)	(40)
ATROPHY, NOS	1 (3%)		1 (3%)
CIRCULATORY SYSTEM			
#MESENTERIC L. NODE	(50)	(46)	(48)
LYMPHANGIECTASIS	1 (2%)	2 (4%)	1 (2%)
#HEART	(49)	(49)	(50)
THROMBUS, MURAL		1 (2%)	1 (2%)
#HEART/ATRIUM	(49)	(49)	(50)
THROMBUS, MURAL		1 (2%)	
#MYOCARDIUM	(49)	(49)	(50)
DEGENERATION, NOS	26 (53%)	16 (33%)	20 (40%)
#PANCREAS	(48)	(48)	(47)
PERIARTERITIS			1 (2%)
#UTERUS	(49)	(49)	(50)
THROMBOSIS, NOS	4 (8%)	1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(49)	(49)	(50)
CYST, NOS		1 (2%)	
CONGESTION, CHRONIC PASSIVE			1 (2%)
FIBROSIS			1 (2%)
NECROSIS, FOCAL		2 (4%)	
METAMORPHOSIS FATTY	7 (14%)	9 (18%)	6 (12%)
CYTOPLASMIC VACUOLIZATION	1 (2%)		
BASOPHILIC CYTO CHANGE	5 (10%)	1 (2%)	7 (14%)
CLEAR-CELL CHANGE		1 (2%)	
ANGIECTASIS			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#LIVER/CENTRILOBULAR	(49)	(49)	(50)
NECROSIS, NOS		1 (2%)	
#BILE DUCT	(49)	(49)	(50)
HYPERPLASIA, NOS	15 (31%)	14 (29%)	15 (30%)
#PANCREAS	(48)	(48)	(47)
FIBROSIS, FOCAL	1 (2%)		
NECROSIS, FOCAL			1 (2%)
#ESOPHAGUS	(45)	(46)	(43)
HYPERKERATOSIS			1 (2%)
#STOMACH	(49)	(49)	(50)
ULCER, NOS	2 (4%)	2 (4%)	3 (6%)
INFLAMMATION, CHRONIC		1 (2%)	
INFLAMMATION, CHRONIC FOCAL			2 (4%)
HYPERPLASIA, BASAL CELL	1 (2%)	3 (6%)	1 (2%)
ACANTHOSIS	1 (2%)	1 (2%)	
#GASTRIC MUCOSA	(49)	(49)	(50)
ULCER, NOS		1 (2%)	
#GASTRIC SUBMUCOSA	(49)	(49)	(50)
INFLAMMATION, FOCAL			1 (2%)
#FORESTOMACH	(49)	(49)	(50)
HYPERPLASIA, BASAL CELL		1 (2%)	
#COLON	(46)	(47)	(50)
EPIDERMAL INCLUSION CYST	1 (2%)		
EDEMA, NOS		1 (2%)	
PARASITISM	4 (9%)	2 (4%)	1 (2%)
HYPERTROPHY, NOS		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(49)	(49)	(50)
MINERALIZATION	1 (2%)		
CYST, NOS	1 (2%)		
INFLAMMATION, CHRONIC FOCAL			1 (2%)
NEPHROSIS, NOS	16 (33%)	13 (27%)	9 (18%)
NEPHROSIS, CHOLEMIC	1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
NECROSIS, MEDULLARY		1 (2%)	
CALCIFICATION, FOCAL	15 (31%)	8 (16%)	12 (24%)
#KIDNEY/CAPSULE	(49)	(49)	(50)
ABSCESS, NOS		1 (2%)	
#RENAL PAPILLA	(49)	(49)	(50)
CALCIFICATION, NOS		1 (2%)	
#URINARY BLADDER	(50)	(49)	(48)
HEMATOMA, NOS		1 (2%)	
INFLAMMATION, CHRONIC		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(50)	(44)	(47)
CYST, NOS	2 (4%)	2 (5%)	12 (26%)
MULTIPLE CYSTS	1 (2%)		1 (2%)
HEMORRHAGIC CYST		1 (2%)	1 (2%)
HYPERPLASIA, NOS	2 (4%)		1 (2%)
HYPERPLASIA, FOCAL	1 (2%)		
HYPERPLASIA, CHROMOPHOBE-CELL		1 (2%)	1 (2%)
VASCULARIZATION	3 (6%)	1 (2%)	1 (2%)
#ADRENAL	(48)	(49)	(50)
HEMORRHAGE			1 (2%)
CALCIFICATION, FOCAL	1 (2%)		
#ADRENAL CORTEX	(48)	(49)	(50)
HEMORRHAGE	1 (2%)		
HYPERPLASIA, NODULAR	2 (4%)	2 (4%)	6 (12%)
DYSPLASIA, NOS		1 (2%)	
#ADRENAL MEDULLA	(48)	(49)	(50)
HYPERPLASIA, NOS	1 (2%)		
HYPERPLASIA, FOCAL			1 (2%)
#THYROID	(49)	(47)	(49)
HYPERPLASIA, C-CELL	7 (14%)	4 (9%)	2 (4%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
GALACTOCELE	12 (24%)	7 (14%)	9 (18%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#UTERUS	(49)	(49)	(50)
HYDROMETRA	1 (2%)	1 (2%)	1 (2%)
HEMORRHAGIC CYST			1 (2%)
INFLAMMATION, NOS			1 (2%)
#UTERUS/ENDOMETRIUM	(49)	(49)	(50)
CYST, NOS			1 (2%)
DECIDUAL ALTERATION, NOS			1 (2%)
#OVARY	(48)	(48)	(50)
CYST, NOS	1 (2%)		
NERVOUS SYSTEM			
#BRAIN	(50)	(50)	(49)
HYDROCEPHALUS, NOS		3 (6%)	2 (4%)
HEMORRHAGE			2 (4%)
SPECIAL SENSE ORGANS			
*EXTERNAL EAR	(50)	(50)	(50)
HEMATOMA, NOS			1 (2%)
MUSCULOSKELETAL SYSTEM			
*SKULL	(50)	(50)	(50)
DEFORMITY, NOS	1 (2%)		
BODY CAVITIES			
*ABDOMINAL VISCERA	(50)	(50)	(50)
CONGESTION, ACUTE			1 (2%)
*PLEURA	(50)	(50)	(50)
EMPYEMA	1 (2%)		
*PERICARDIUM	(50)	(50)	(50)
INFLAMMATION, ACUTE	1 (2%)		
INFLAMMATION ACUTE AND CHRONIC			1 (2%)
*MESENTERY	(50)	(50)	(50)
NECROSIS, FAT	4 (8%)	1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(50)
HEMORRHAGE		1 (2%)	
GRANULOMA, FOREIGN BODY	1 (2%)		
CALCIFICATION, FOCAL			1 (2%)
OMENTUM			
NECROSIS, FAT			3
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

APPENDIX D

Summary of the Incidence of Nonneoplastic Lesions in Mice Fed Diets Containing Gum Arabic

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE FED DIETS CONTAINING GUM ARABIC

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	49	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	49	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(49)	(50)	(50)
ULCER, NOS	1 (2%)		
INFLAMMATION, FOCAL		1 (2%)	
ABSCESS, NOS	1 (2%)		
INFLAMMATION, CHRONIC FOCAL	1 (2%)		
*SUBCUT TISSUE	(49)	(50)	(50)
HEMORRHAGE		1 (2%)	
INFLAMMATION, NOS		1 (2%)	
ABSCESS, NOS			1 (2%)
GRANULOMA, NOS			1 (2%)
INFECTION, FUNGAL			1 (2%)
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(49)	(50)	(50)
HYPERPLASIA, LYMPHOID		1 (2%)	
HEMATOPOIESIS			1 (2%)
#SPLEEN	(47)	(48)	(49)
CONGESTION, NOS	1 (2%)	1 (2%)	2 (4%)
HEMORRHAGE			1 (2%)
NECROSIS, NOS			1 (2%)
INFARCT, NOS		1 (2%)	
HYPERPLASIA, LYMPHOID		1 (2%)	
HEMATOPOIESIS	2 (4%)	2 (4%)	2 (4%)
#MEDIASTINAL L.NODE	(42)	(45)	(48)
HYPERPLASIA, NOS			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#MESENTERIC L. NODE	(42)	(45)	(48)
CONGESTION, NOS	18 (43%)	20 (44%)	24 (50%)
HEMORRHAGE		1 (2%)	
HEMORRHAGIC CYST	2 (5%)		
HYPERPLASIA, LYMPHOID			1 (2%)
#THYMUS	(15)	(21)	(23)
CYST, NOS			1 (4%)
CIRCULATORY SYSTEM			
*MULTIPLE ORGANS	(49)	(50)	(50)
PERIVASCULITIS	1 (2%)		
#LUNG	(49)	(49)	(50)
PERIVASCULITIS			1 (2%)
#AURICULAR APPENDAGE	(49)	(48)	(50)
THROMBOSIS, NOS			1 (2%)
#MYOCARDIUM	(49)	(48)	(50)
DEGENERATION, NOS	2 (4%)	1 (2%)	2 (4%)
*MESENTERY	(49)	(50)	(50)
PERIARTERITIS	1 (2%)		
DIGESTIVE SYSTEM			
#LIVER	(49)	(49)	(50)
NECROSIS, FOCAL			2 (4%)
METAMORPHOSIS FATTY			1 (2%)
BASOPHILIC CYTO CHANGE	1 (2%)		
ANGIECTASIS			1 (2%)
#PANCREAS	(45)	(46)	(49)
DILATATION/DUCTS		1 (2%)	
#STOMACH	(46)	(47)	(48)
INFLAMMATION ACUTE AND CHRONIC		1 (2%)	
INFLAMMATION, CHRONIC FOCAL	1 (2%)		
ATYPIA, NOS		1 (2%)	1 (2%)
HYPERPLASIA, BASAL CELL	1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HYPERKERATOSIS	4 (9%)	1 (2%)	
ACANTHOSIS	1 (2%)	1 (2%)	1 (2%)
#GASTRIC MUCOSA	(46)	(47)	(48)
ATYPIA, NOS			1 (2%)
METAPLASIA, SQUAMOUS	1 (2%)	1 (2%)	2 (4%)
#GASTRIC SUBMUCOSA	(46)	(47)	(48)
INFLAMMATION, ACUTE	1 (2%)		
#COLON	(41)	(44)	(43)
PARASITISM	1 (2%)		
URINARY SYSTEM			
#KIDNEY	(48)	(50)	(50)
HYDRONEPHROSIS		2 (4%)	
PYELONEPHRITIS, NOS		1 (2%)	
PYELONEPHRITIS, FOCAL			1 (2%)
ABSCESS, NOS			1 (2%)
NEPHROPATHY	1 (2%)	2 (4%)	2 (4%)
AMYLOID, NOS			2 (4%)
CALCIFICATION, FOCAL	1 (2%)		
ATROPHY, FOCAL	2 (4%)		
#URINARY BLADDER	(46)	(47)	(50)
CALCULUS, NOS	1 (2%)	3 (6%)	
INFLAMMATION ACUTE AND CHRONIC		1 (2%)	
HYPERPLASIA, EPITHELIAL		1 (2%)	
*URETHRA	(49)	(50)	(50)
CALCULUS, NOS	1 (2%)		
*PROSTATIC URETHRA	(49)	(50)	(50)
CALCULUS, NOS			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(40)	(36)	(38)
HYPERPLASIA, FOCAL		2 (6%)	
#ADRENAL CORTEX	(45)	(48)	(47)
HYPERPLASIA, NODULAR	1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, FOCAL		1 (2%)	
#THYROID	(45)	(46)	(49)
HYPERPLASIA, FOLLICULAR-CELL		1 (2%)	
#PANCREATIC ISLETS	(45)	(46)	(49)
HYPERPLASIA, NOS	1 (2%)		
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(49)	(50)	(50)
CYSTIC DUCTS	1 (2%)		
INFLAMMATION, NOS		1 (2%)	
ABSCESS, NOS		2 (4%)	1 (2%)
#PROSTATE	(44)	(43)	(44)
INFLAMMATION ACUTE AND CHRONIC		1 (2%)	
*SEMINAL VESICLE	(49)	(50)	(50)
INFLAMMATION ACUTE AND CHRONIC		1 (2%)	
#TESTIS	(47)	(49)	(49)
MINERALIZATION	1 (2%)		
ATROPHY, FOCAL	2 (4%)		1 (2%)
NERVOUS SYSTEM			
#SUBARACHNOID SPACE	(46)	(49)	(49)
HEMORRHAGE	1 (2%)		
#BRAIN	(46)	(49)	(49)
CALCIFICATION, FOCAL	17 (37%)	15 (31%)	15 (31%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*ABDOMINAL CAVITY	(49)	(50)	(50)
NECROSIS, FAT	2 (4%)		
ALL OTHER SYSTEMS			
TAIL			
NECROSIS, HEMORRHAGIC			1
LEG			
ULCER, NOS		1	
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1	6	1
AUTO/NECROPSY/HISTO PERF	2		
AUTOLYSIS/NO NECROPSY	1		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE FED DIETS CONTAINING GUM ARABIC

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING	1		
ANIMALS NECROPSIED	49	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	49	50	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(48)	(49)	(50)
EDEMA, NOS			1 (2%)
HEMORRHAGE			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(49)	(50)	(50)
HYPERPLASIA, LYMPHOID			1 (2%)
HEMATOPOIESIS			1 (2%)
#BONE MARROW	(47)	(48)	(47)
FIBROSIS, FOCAL	33 (70%)	39 (81%)	30 (64%)
HYPERPLASIA, HEMATOPOIETIC	3 (6%)		
#SPLEEN	(47)	(48)	(49)
CONGESTION, NOS	1 (2%)		3 (6%)
HYPERPLASIA, LYMPHOID		1 (2%)	2 (4%)
HEMATOPOIESIS	2 (4%)	2 (4%)	2 (4%)
#LYMPH NODE	(45)	(46)	(41)
HEMATOPOIESIS	1 (2%)		
#MANDIBULAR L. NODE	(45)	(46)	(41)
CONGESTION, NOS	1 (2%)		
#MEDIASTINAL L.NODE	(45)	(46)	(41)
INFLAMMATION, CHRONIC	1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#ABDOMINAL LYMPH NODE	(45)	(46)	(41)
INFLAMMATION ACUTE AND CHRONIC		1 (2%)	
#MESENTERIC L. NODE	(45)	(46)	(41)
CONGESTION, NOS	3 (7%)	2 (4%)	1 (2%)
#LIVER	(49)	(50)	(50)
HEMATOPOIESIS			1 (2%)
CIRCULATORY SYSTEM			
*MULTIPLE ORGANS	(49)	(50)	(50)
PERIVASCULITIS			1 (2%)
*ABDOMINAL CAVITY	(49)	(50)	(50)
PERIVASCULITIS	1 (2%)		
#LUNG	(48)	(49)	(50)
EMBOLISM, NOS		1 (2%)	
PERIVASCULITIS			1 (2%)
#PANCREAS	(47)	(47)	(45)
LYMPHANGIECTASIS	1 (2%)		
#COLON	(42)	(45)	(44)
PERIARTERITIS		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(49)	(50)	(50)
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	
NECROSIS, FOCAL	1 (2%)	2 (4%)	1 (2%)
METAMORPHOSIS FATTY	1 (2%)	2 (4%)	3 (6%)
CYTOPLASMIC VACUOLIZATION	1 (2%)		
*GALLBLADDER	(49)	(50)	(50)
INFLAMMATION ACUTE AND CHRONIC	1 (2%)		
#PANCREAS	(47)	(47)	(45)
DILATATION/DUCTS	1 (2%)	3 (6%)	
INFLAMMATION, NOS	1 (2%)		
#PANCREATIC ACINUS	(47)	(47)	(45)
ATROPHY, NOS		3 (6%)	1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#STOMACH	(45)	(46)	(49)
ULCER, NOS	2 (4%)		
HYPERPLASIA, BASAL CELL		1 (2%)	3 (6%)
HYPERKERATOSIS	4 (9%)	5 (11%)	
ACANTHOSIS	2 (4%)	1 (2%)	5 (10%)
#GASTRIC MUCOSA	(45)	(46)	(49)
ABSCESS, NOS		1 (2%)	
ATYPIA, NOS		1 (2%)	3 (6%)
#GASTRIC SUBMUCOSA	(45)	(46)	(49)
INFLAMMATION, ACUTE FOCAL	1 (2%)		
INFLAMMATION, CHRONIC FOCAL	1 (2%)		
#FORESTOMACH	(45)	(46)	(49)
INFLAMMATION, CHRONIC		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(49)	(48)	(48)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
NEPHROPATHY		1 (2%)	1 (2%)
GLOMERULOSCLEROSIS, NOS		1 (2%)	
CALCIFICATION, FOCAL	1 (2%)		
ATROPHY, FOCAL		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(39)	(41)	(40)
HYPERPLASIA, NODULAR	1 (3%)		
HYPERPLASIA, NOS	1 (3%)		
HYPERPLASIA, FOCAL		1 (2%)	2 (5%)
HYPERPLASIA, CHROMOPHOBE-CELL	1 (3%)	1 (2%)	
ANGIECTASIS	1 (3%)		
#ADRENAL CORTEX	(48)	(44)	(43)
METAMORPHOSIS FATTY		1 (2%)	
#ADRENAL MEDULLA	(48)	(44)	(43)
HYPERPLASIA, FOCAL	2 (4%)		2 (5%)
#THYROID	(45)	(45)	(43)
HYPERPLASIA, C-CELL			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM			
#UTERUS	(48)	(49)	(49)
HYDROMETRA	6 (13%)	4 (8%)	5 (10%)
INFLAMMATION, NOS	1 (2%)		1 (2%)
PYOMETRA			2 (4%)
ABSCESS, NOS		1 (2%)	
ATROPHY, NOS	1 (2%)		
#UTERUS/ENDOMETRIUM	(48)	(49)	(49)
HYPERPLASIA, CYSTIC	24 (50%)	25 (51%)	25 (51%)
METAPLASIA, SQUAMOUS		1 (2%)	1 (2%)
#UTERUS/MYOMETRIUM	(48)	(49)	(49)
INFLAMMATION, ACUTE			1 (2%)
#TUBO OVARIAN COMBINE	(48)	(49)	(49)
ABSCESS, NOS		1 (2%)	1 (2%)
#OVARY	(40)	(40)	(43)
CYST, NOS	2 (5%)	3 (8%)	4 (9%)
MULTIPLE CYSTS		1 (3%)	
HEMORRHAGE	1 (3%)		
HEMORRHAGIC CYST		1 (3%)	
INFLAMMATION, NOS	1 (3%)		
ABSCESS, NOS			1 (2%)
NERVOUS SYSTEM			
#BRAIN/MENINGES	(49)	(49)	(50)
INFLAMMATION, NOS	1 (2%)		
#BRAIN	(49)	(49)	(50)
CALCIFICATION, FOCAL	13 (27%)	11 (22%)	16 (32%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*ABDOMINAL CAVITY	(49)	(50)	(50)
HEMORRHAGE			1 (2%)
ABSCESS, NOS	1 (2%)		
NECROSIS, FAT	2 (4%)		
*PERITONEUM	(49)	(50)	(50)
HEMOPERITONEUM	1 (2%)		
INFLAMMATION, ACUTE	1 (2%)		
*PLEURA	(49)	(50)	(50)
INFLAMMATION, NOS			1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(49)	(50)	(50)
INFLAMMATION, ACUTE FOCAL	1 (2%)		
OMENTUM			
NECROSIS, FAT			1
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	2		2
ANIMAL MISSING/NO NECROPSY	1		
AUTO/NECROPSY/HISTO PERF	1		1

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

APPENDIX E

Analysis of Gum Arabic (Lot No. 54-36431)
Midwest Research Institute

APPENDIX E

Analysis of Gum Arabic (Lot No. 54-36431)
Midwest Research Institute

A. MELTING POINT

Determined	Literature Values
m.p.: 210° to 300°C (visual, capilliary) Exotherm beginning at 274°C, decomp (Dupont 900 DTA)	No literature value found

B. THIN-LAYER CHROMATOGRAPHY OF ACID HYDROLYSIS PRODUCTS (Varma et al., 1973)

Plates Silica Gel 60 F-254

Ref. Standard: D-galactose
L-arabinose
L-rhamnose

Amount spotted: 42 μg

Visualization: 0.5% potassium permanganate in 1 N sodium hydroxide

System 1: n-Butanol:water: Acetic acid (50:20:10)	System 2: n-Butanol:pyridine: water (30:20:15)
R_f: 0.43 (rhamnose) 0.24 (arabinose) 0.18 (galactose) 0.04 (possibly glucuronic acid)	R_f: 0.63, 0.45, 0.36, 0.19
R_{st}: 2.5, 1.4, 1.1, 0.24 relative to D-galactose 1.7, 0.96, 0.72, 0.16 relative to L-arabinose 0.98, 0.55, 0.41, 0.09 relative to L-rhamnose	R_{st}: 1.7, 1.2, 0.97, 0.51 relative to D-galactose 1.4, 0.98, 0.78, 0.41 relative to L-arabinose 0.97, 0.69, 0.55, 0.29 relative to L-rhamnose

C. WATER ANALYSIS (Karl Fisher)

12.3 ± 0.8 (δ)%

D. CATION ANALYSIS

Na - $\leq 0.08\%$
K - $0.70 \pm 0.02\%$
Mg - $0.20 \pm 0.01\%$
Ca - $0.66 \pm 0.01\%$

E. TITRATION BY PERIODATE OXIDATION

Modification of USP Assay for Mannitol (USP, 1970): Samples were dissolved in 25 ml of water in 250 ml volumetric flasks and left at room temperature for 65 hours. The solutions were then boiled for 55 minutes on a hot plate. The flasks were cooled and diluted to volume with water. Aliquots (5 ml) were transferred to 125-ml Erlenmeyer flasks and 50.0 ml potassium periodate/sulfuric acid solution was added. The sample and the blank were heated on a steam bath for 25 hours. Potassium iodide was added and the samples were titrated with sodium thiosulfate.

Results: $80.8\% \pm 2.4$ (δ)% as compared with a glucose standard. (The assumption was made that 5 moles of periodate were needed for each monomer unit of the polysaccharide).

F. SPECTRAL DATA

(1) Infrared:

Instrument: Beckman IR-12 Cell: 1% in potassium bromide Results: See Figure 5.	Consistent with literature spectrum (McNulty, 1960)

(2) Ultraviolet/Visible:

Instrument: No absorbance between 200 and 350 nm (ultraviolet range) or between 350 and 800 nm (visible range) Concentration: 0.1 mg/ml Solvent: Water	No literature reference found

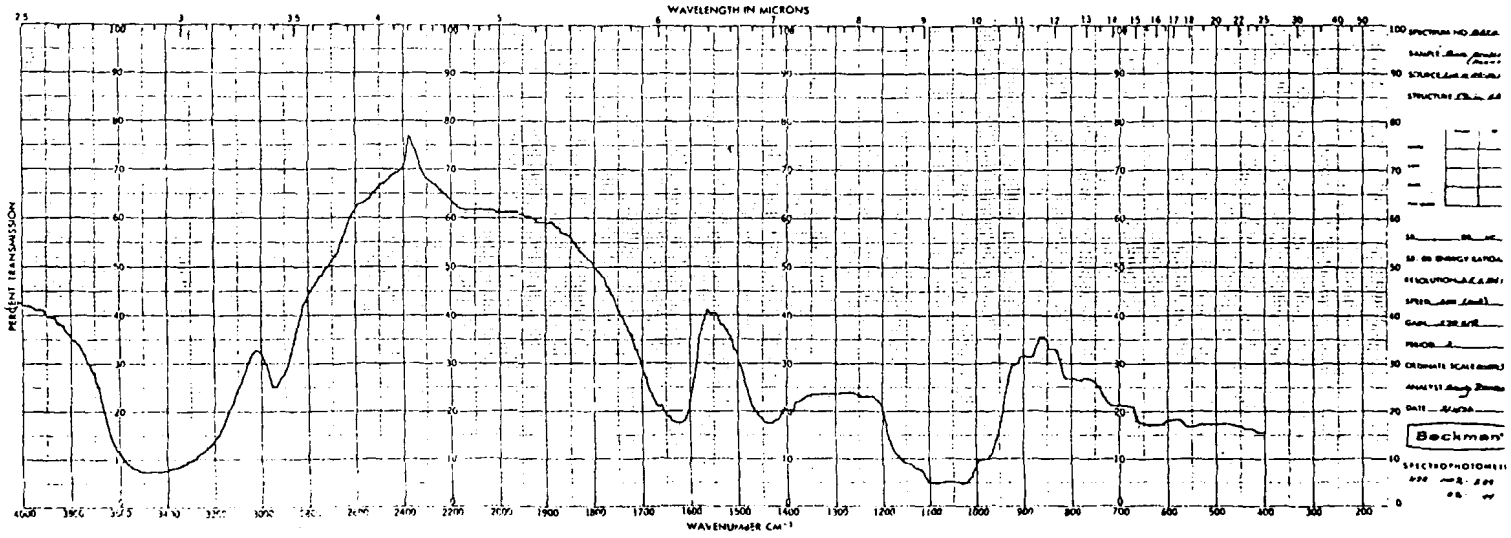

Figure 5. Infrared Absorption Spectrum of Gum Arabic (Lot No. 54-36431)

APPENDIX F

Analysis of Gum Arabic (Lot No. 54-77890)
Midwest Research Institute

APPENDIX F

Analysis of Gum Arabic (Lot No. 54-77890)
Midwest Research Institute

A. THIN-LAYER CHROMATOGRAPHY OF ACID HYDROLYSIS PRODUCTS (Varma et al., 1973)

Plates: Silica Gel 60 F-254

Ref. Standards: D-galactose
L-arabinose
L-rhamnose

Amount Spotted: 40μg, 2μg/μl in H_2O:methanol (25:75)

Visualization: 0.5% potassium permanganate in 1 N sodium hydroxide

System 1: n-Butanol:acetic acid:water (63:12:25)

System 2: n-Butanol:pyridine: water (46:31:23)

R_f: 0.04 (trace), 0.20, 0.26, 0.45 (trace)

R_f: 0.19, 0.49, 0.57, 0.71

R_{st}: 0.21, 1.02, 1.34
2.31 relative to D-galactose
0.16, 0.78, 1.02, 1.76
relative to L-arabinose
0.09, 0.45, 0.59, 1.02
relative to L-rhamnose

R_{st}: 0.39, 1.02, 1.18, 1.47
relative to D-galactose
0.34, 0.88, 1.02, 1.27
relative to L-arabinose
0.27, 0.71, 0.84, 1.03
relative to L-rhamnose

B. WATER ANALYSIS
(Karl Fisher)

9.0 ± 0.9 (δ)%

C. TITRATION BY PERIODATE OXIDATION (USP, 1970)

Modification of U.S.P. Assay for Mannitol

Samples were dissolved in 25 ml concentrated sulfuric acid and 150 ml water in 250-ml volumetric flasks and left at room temperature for 18 hours.

The solutions were then boiled on a hot plate until they started to discolor. All samples began to discolor before 15 minutes. The flasks were cooled and diluted to volume with water. Aliquots (5 ml) were transferred to 125-ml Erlenmeyer flasks and 50.0 ml potassium periodate/sulfuric acid solutions was added. Each sample and a blank were heated on a steam bath for 2.5 hours. Potassium iodide was added and the samples titrated with sodium thiosulfate. The assumption was made that each monomer unit reacted with 5 moles of periodate.

Results: 85.5 ± 2.1 (δ)%

D. SPECTRAL DATA

Infrared Spectrum

Instrument: Beckman IR-12

Consistent with literature spectrum (McNulty, 1960)

Cell: Thin film

Results: See Figure 6

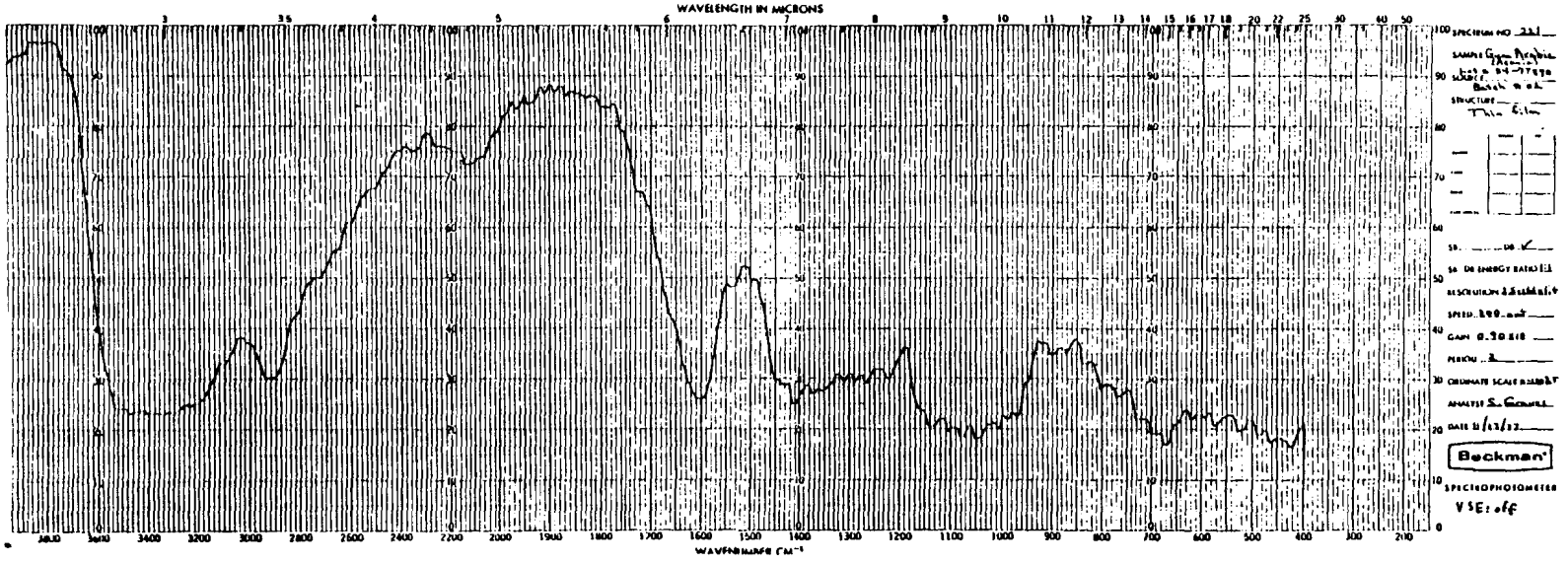

Figure 6. Infrared Absorption Spectrum of Gum Arabic (Lot No. 54-77890)

APPENDIX G

Feed Consumption by Rats and Mice Receiving Gum Arabic

Table G1. Feed Consumption by Male Rats Receiving Gum Arabic

Week	Control Grams Feed/ Day(a)	Low Grams Feed/ Day(a)	Low Low/ Control (b)	High Grams Feed/ Day(a)	High High/ Control (b)
4	24.3	24.6	1.0	9.4	0.4
8	24.0	22.3	0.9	22.4	0.9
12	20.4	19.1	0.9	16.4	0.8
16	18.7	17.3	0.9	17.1	0.9
20	20.6	19.0	0.9	18.3	0.9
24	21.7	20.0	0.9	20.6	0.9
28	16.0	19.3	1.2	19.3	1.2
32	21.7	16.4	0.8	19.1	0.9
36	23.0	22.4	1.0	20.9	0.9
40	22.0	20.0	0.9	20.7	0.9
44	23.7	21.1	0.9	21.4	0.9
48	23.1	21.1	0.9	21.6	0.9
52	22.1	21.4	1.0	20.9	0.9
56	25.4	22.6	0.9	21.7	0.9
60	24.9	22.9	0.9	22.6	0.9
64	27.1	23.9	0.9	22.7	0.8
68	23.9	23.1	1.0	22.1	0.9
72	17.9	19.6	1.1	20.3	1.1
76	23.0	20.7	0.9	21.9	1.0
80	21.0	20.6	1.0	19.0	0.9
84	21.1	18.1	0.9	17.9	0.8
88	20.0	18.3	0.9	13.0	0.7
92	21.7	20.9	1.0	18.6	0.9
96	27.0	24.0	0.9	20.0	0.7
100	18.9	20.9	1.1	17.6	0.9
Mean	22.1	20.8	1.0	19.4	0.9
SD (c)	2.7	2.1	0.1	3.1	0.1
CV (d)	12.2	10.1	10.0	16.0	11.1

(a) Grams of feed consumed per animal per day.
(b) Ratio of feed consumed per day for the dosed group to that for the controls.
(c) Standard deviation.
(d) (Standard deviation/Mean) x 100.

Table G2. Feed Consumption by Female Rats Receiving Gum Arabic

Week	Control Grams Feed/ Day(a)	Low Grams Feed/ Day(a)	Low Low/ Control (b)	High Grams Feed/ Day(a)	High High/ Control (b)
4	15.9	16.7	1.1	15.9	1.0
8	14.3	14.1	1.0	13.3	0.9
12	16.9	14.4	0.9	15.0	0.9
16	17.9	14.9	0.8	16.1	0.9
20	19.4	16.0	0.8	15.3	0.8
24	18.0	15.7	0.9	16.6	0.9
28	18.1	15.9	0.9	16.0	0.9
29	17.0	17.8	1.0	15.4	0.9
32	15.9	19.7	1.2	16.3	1.0
36	19.0	16.7	0.9	17.6	0.9
40	20.9	17.1	0.8	17.6	0.8
44	17.7	15.7	0.9	16.6	0.9
48	18.0	15.3	0.8	15.4	0.9
52	19.6	15.3	0.8	16.1	0.8
56	20.7	16.1	0.8	15.0	0.7
60	19.9	16.6	0.8	15.9	0.8
64	21.9	17.6	0.8	17.4	0.8
68	20.9	16.9	0.8	17.7	0.8
72	18.6	17.9	1.0	15.6	0.8
76	22.1	17.7	0.8	17.4	0.8
80	20.0	16.3	0.8	16.3	0.8
84	20.0	16.4	0.8	17.7	0.9
88	18.1	15.3	0.8	15.6	0.9
92	18.1	16.6	0.9	16.6	0.9
96	19.0	17.3	0.9	18.6	1.0
100	18.3	17.1	0.9	16.3	0.9
Mean	18.7	16.4	0.9	16.3	0.9
SD (c)	1.9	1.2	0.1	1.1	0.1
CV (d)	10.2	7.3	11.1	6.7	11.1

(a) Grams of feed consumed per animal per day.
(b) Ratio of feed consumed per day for the dosed group to that for the controls.
(c) Standard deviation.
(d) (Standard deviation/Mean) x 100.

Table G3. Feed Consumption by Male Mice Receiving Gum Arabic

Week	Control Grams Feed/ Day(a)	Low Grams Feed/ Day(a)	Low Low/ Control (b)	High Grams Feed/ Day(a)	High High/ Control (b)
4	8.4	7.3	0.9	7.4	0.9
8	8.0	6.9	0.9	7.6	1.0
12	7.1	6.6	0.9	7.3	1.0
16	7.7	7.0	0.9	6.7	0.9
20	5.1	4.4	0.9	4.1	0.8
24	7.1	6.6	0.9	6.6	0.9
28	7.6	6.1	0.8	6.6	0.9
32	7.3	6.0	0.8	6.0	0.8
36	7.6	9.0	1.2	5.9	0.8
40	6.9	6.6	1.0	6.0	0.9
44	6.0	5.9	1.0	6.0	1.0
48	5.9	5.6	0.9	5.9	1.0
52	5.4	8.1	1.5	5.9	1.1
56	6.1	5.6	0.9	5.1	0.8
60	7.1	5.9	0.8	5.7	0.8
64	7.6	6.9	0.9	7.3	1.0
68	6.9	5.9	0.9	5.7	0.8
72	9.1	6.0	0.7	6.6	0.7
76	8.9	6.3	0.7	6.0	0.7
80	9.3	6.3	0.7	6.4	0.7
84	6.1	6.1	1.0	6.3	1.0
88	6.4	5.6	0.9	5.6	0.9
92	6.9	5.6	0.8	6.6	1.0
96	7.4	5.9	0.8	6.4	0.9
100	9.6	6.9	0.7	6.6	0.7
Mean	7.3	6.3	0.9	6.2	0.9
SD (c)	1.2	0.9	0.2	0.7	0.1
CV (d)	16.4	14.3	22.2	11.3	11.1

(a) Grams of feed consumed per animal per day.
(b) Ratio of feed consumed per day for the dosed group to that for the controls.
(c) Standard deviation.
(d) (Standard deviation/Mean) x 100.

Table G4. Feed Consumption by Female Mice Receiving Gum Arabic

Week	Control Grams Feed/ Day(a)	Low Grams Feed/ Day(a)	Low Low/ Control (b)	High Grams Feed/ Day(a)	High High/ Control (b)
4	9.0	7.6	0.8	7.4	0.8
8	10.0	7.9	0.8	8.1	0.8
12	10.6	10.1	1.0	8.6	0.8
16	9.4	8.7	0.9	8.7	0.9
20	6.1	5.3	0.9	5.1	0.8
24	9.7	9.3	1.0	8.7	0.9
28	10.1	8.7	0.9	8.1	0.8
32	9.6	8.3	0.9	7.3	0.8
36	8.7	8.0	0.9	8.4	1.0
40	9.6	8.4	0.9	8.3	0.9
44	7.1	4.4	0.6	7.3	1.0
48	8.1	6.7	0.8	8.1	1.0
52	7.9	7.1	0.9	6.9	0.9
56	7.4	7.4	1.0	7.6	1.0
60	7.0	6.9	1.0	7.1	1.0
64	9.0	8.7	1.0	8.9	1.0
68	8.9	7.9	0.9	8.1	0.9
72	10.3	7.6	0.7	7.3	0.7
76	10.9	8.9	0.8	8.1	0.7
80	10.1	8.9	0.9	9.0	0.9
84	9.1	8.6	0.9	8.1	0.9
88	7.6	6.7	0.9	6.0	0.8
92	7.1	7.3	1.0	6.9	1.0
96	9.1	7.9	0.9	8.1	0.9
100	10.0	8.0	0.8	8.7	0.9
Mean	8.9	7.8	0.9	7.8	0.9
SD (c)	1.3	1.2	0.1	0.9	0.1
CV (d)	14.6	15.4	11.1	11.5	11.1

(a) Grams of feed consumed per animal per day.
(b) Ratio of feed consumed per day for the dosed group to that for the controls.
(c) Standard deviation.
(d) (Standard deviation/Mean) x 100.

☆U.S. GOVERNMENT PRINTING OFFICE: 1982-361-132:566

SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 1 March 2017

doi: 10.2903/j.efsa.2017.4741

Re-evaluation of acacia gum (E 414) as a food additive

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS), Alicja Mortensen, Fernando Aguilar, Riccardo Crebelli, Alessandro Di Domenico, Maria Jose Frutos, Pierre Galtier, David Gott, Ursula Gundert-Remy, Claude Lambré, Jean-Charles Leblanc, Oliver Lindtner, Peter Moldeus, Pasquale Mosesso, Agneta Oskarsson, Dominique Parent-Massin, Ivan Stankovic, Ine Waalkens-Berendsen, Rudolf Antonius Woutersen, Matthew Wright, Maged Younes, Leon Brimer, Anna Christodoulidou, Federica Lodi, Alexandra Tard and Birgit Dusemund

Abstract

The Panel on Food Additives and Nutrient Sources added to Food (ANS) provides a scientific opinion re-evaluating the safety of acacia gum (E 414) as a food additive. In the EU, acacia gum has not been formally evaluated by the Scientific Committee for Food (SCF), and therefore, no ADI has been allocated. However, it was accepted for use in weaning food (SCF, 1991). In 1999, the SCF considered 'that the use of acacia gum/gum arabic in coatings for nutrient preparations containing trace elements is acceptable provided carry-over levels in infant formulae, follow-on formulae or FSMP do not exceed 10 mg/kg'. Acacia gum was evaluated by JECFA in 1982 and 1990 and the specifications were amended in 1998. Based on the lack of adverse effects in the available toxicity studies, an ADI 'not specified' was allocated. Following the conceptual framework for the risk assessment of certain food additives re-evaluated under Commission Regulation (EU) No 257/2010, the Panel considered that adequate exposure and toxicity data were available. Acacia gum is unlikely to be absorbed intact and is slightly fermented by intestinal microbiota. No adverse effects were reported in subchronic and carcinogenicity studies at the highest dose tested and there is no concern with respect to the genotoxicity. Oral daily intake of a large amount of acacia gum up to 30,000 mg acacia gum/person per day (approximately equivalent 430 mg acacia gum/kg bw per day) for up to 18 days was well tolerated in adults but some individuals experienced flatulence which was considered by the Panel as undesirable but not adverse effect. The Panel concluded that there is no need for a numerical ADI for acacia gum (E 414), and there is no safety concern for the general population at the refined exposure assessment of acacia gum (E 414) as a food additive.

Keywords: Acacia gum (E 414), gum arabic, CAS Registry Number 9000-01-5, food additive

Requestor: European Commission

Question number: EFSA-Q-2011-00513

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, Agneta Oskarsson, Dominique Parent-Massin, Ivan Stankovic, Ine Waalkens-Berendsen, Rudolf Antonius Woutersen, Matthew Wright and Maged Younes.

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

Suggested citation: EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), Mortensen A, Aguilar F, Crebelli R, Di Domenico A, Frutos MJ, Galtier P, Gott D, Gundert-Remy U, Lambré C, Leblanc J-C, Lindtner O, Moldeus P, Mosesso P, Oskarsson A, Parent-Massin D, Stankovic I, Waalkens-Berendsen I, Woutersen RA, Wright M, Younes M, Brimer L, Christodoulidou A, Lodi F, Tard A and Dusemund B, 2017. Scientific Opinion on the re-evaluation of acacia gum (E 414) as a food additive. EFSA Journal 2017;15(4):4741, 51 pp. doi:10.2903/j.efsa.2017.4741

ISSN: 1831-4732

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

Summary

Following a request from the European Commission, the Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to re-evaluate the safety of acacia gum (E 414) when used as a food additive.

The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations and reviews, additional literature that has come available since then and the data available following a public call for data. The Panel noted that not all original studies on which previous evaluations were based were available for re-evaluation by the Panel.

Acacia Gum (E 414) is authorised as a food additive in the European Union (EU) in accordance with Annex II and Annex III to Regulation (EC) No 1333/2008 on food additives.

In the EU, acacia gum has not been formally evaluated by the Scientific Committee for Food (SCF) and therefore no acceptable daily intake (ADI) has been allocated. However, it was accepted for use in weaning food (SCF, 1991). In 1999, the SCF considered 'that the use of acacia gum/gum arabic in coatings for nutrient preparations containing trace elements is acceptable provided carry-over levels in infant formulae, follow-on formulae or FSMP do not exceed 10 mg/kg' (SCF, 1999). Acacia gum was evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1982 and 1990 (JECFA, 1982, 1990). Based on the lack of adverse effects in the available toxicity studies, an ADI 'not specified' was allocated. It was stressed that the evaluation covered only acacia gum from *Acacia senegal* and closely related species; the specifications were amended in 1998 to cover also acacia gum from *Acacia seyal* (JECFA, 1998).

Acacia gum is a dried exudation obtained from the stems and branches of natural strains of *A. senegal* (L.) Willdenow or closely related species of *Acacia* (family Leguminosae) (JECFA, 2006).

Specifications for acacia gum (E 414) have been defined in Commission Regulation (EU) 231/2012. The Panel noted that according to the EC specifications it is not clear which are the closely related species of *Acacia*, while in the JECFA specifications (JECFA, 2006), it is indicated that gum arabic (acacia gum) can be obtained from *A. senegal* (L.) Willdenow or *A. seyal* (family Leguminosae). The Panel noted that the EC specifications do not limit the protein content which according to Phillips et al. (2008) can be between 0.13% and 10.4%. According to industry (Documentation provided to EFSA, n.5), contents of proteins were in a range from 0.99% to 2.70% as determined in three samples analysed in duplicate. The Panel agreed with the proposal by interested parties to include a limit for protein content of 3.5% in the EC specifications.

Because of both the botanical origin and the polysaccharidic nature of gums, they can be a substrate of microbiological contamination and of field and storage fungal development. The latter has been recently demonstrated by the mycotoxin contaminations of gums (Zhang et al., 2014). The Panel noted that the microbiological specifications for polysaccharidic thickening agents, such as gums, should be harmonised and that for acacia gum criteria for total aerobic microbial count (TAMC) and total combined yeasts and moulds count (TYMC) should be included into the EU specifications.

Regarding the possible presence of nanoparticles in the dry powder of acacia gum resulting from the manufacturing process, the Panel considered that the material used for toxicological testing would contain this nanofraction, if present. In addition, the Panel noted that contact of acacia gum with any liquids (in food or biological fluids) will result in an increase of the particle size.

The *in vitro* degradation and the *in vivo* digestibility of acacia gum have been investigated in animals and humans models and in a human study. The Panel considered that these data indicated that acacia gum would be not absorbed intact but fermented by enteric bacteria in humans. The rate of hydrolysis in the gastrointestinal tract in humans is unknown; however, the Panel considered that acacia gum is unlikely to be absorbed intact, and that the limited extent of its fermentation would lead to products such as short-chain fatty acids (SCFA) which were considered of no safety concern by the Panel.

Among other studies, the subchronic (13 weeks) oral toxicity of acacia gum was investigated by Anderson et al. (1982). The animals received acacia gum in their diet and the study was conducted in two consecutive experimental phases. In the first one, the rats were given doses ranging from 0 to about 5,000 mg acacia gum/kg body weight (bw) per day, and in the second phase, they received 0 or 14,000 mg acacia gum/kg bw per day. The Panel noted that these two studies were done independently and that merging their data may not be straightforward. The Panel considered that no toxicological effect was observed in these studies by Anderson et al. (1982). From the first study, no adverse effects have been identified up to 5,220 and 5,310 mg acacia gum/kg bw per day in male and female, respectively, the highest dose tested.

Overall, the short-term and subchronic administration of oral doses up to 5,000 mg acacia gum/kg bw per day to rats and 20,000 mg acacia gum/kg bw per day to mice, the highest doses tested, did not induce any biologically relevant adverse effects. In some studies, caecal enlargement was observed. The Panel considered that an increased caecum weight in animals fed high amounts of carbohydrates is considered as a physiological response to an increased fermentation by the intestinal microbiota.

Based on the data available, the Panel considered that there is no concern with respect to the genotoxicity of acacia gum.

No chronic toxicity studies according to OECD guidelines (452) or equivalent have been identified.

Acacia gum was tested for carcinogenicity in rats and mice receiving diets containing 2.5% and 5% acacia gum in the feed for 103 weeks equivalent to 1,250 and 2,500 mg acacia gum/kg bw per day in rats, and 3,750 and 7,500 mg acacia gum/kg bw per day in mice (NTP, 1982; Melnick et al., 1983). From this study, the Panel considered that acacia gum is not of concern with respect to carcinogenicity.

In a dietary combined fertility and developmental toxicity study in rats (Collins et al., 1987), a no observed adverse effect level (NOAEL) of 10,647 mg acacia gum/kg bw per day for reproductive, developmental and parental effects was identified, the highest dose tested. In addition, other reproductive studies in rats showed no effects at the highest dose tested (Morseth and Ihara (1989a), Huynh et al., 2000). In the identically performed prenatal developmental tests with acacia gum by gavage in mice, rats and hamsters (FDRL, 1972b), 1,600 mg/kg bw per day (the highest dose tested) showed no dose-related developmental effects.

No case reports on allergic reaction after oral exposure to acacia gum could be identified by the Panel.

In humans, the repeated oral daily intake of a large amount of acacia gum up to 30 g (approx. 430 mg acacia gum/kg bw per day) for up to 18 days was well tolerated and had only a minimum effect on stool weight and decrease in serum cholesterol. Some individuals experienced flatulence which was considered by the Panel as undesirable but not adverse.

Acacia (E 414) is authorised in a wide range of foods. The Panel did not identify brand loyalty to a specific food category, and therefore, the Panel considered that the non-brand-loyal scenario covering the general population was the more appropriate and realistic scenario for risk characterisation because it is assumed that the population would probably be exposed long-term to the food additive present at the mean reported use in processed food.

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

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

The refined estimates are based on 31 out of 76 food categories in which acacia gum (E 414) is authorised. The Panel considered that the uncertainties identified would, in general, result in an overestimation of the exposure to acacia gum (E 414) as a food additive in European countries for the refined scenario if it is considered that the food additive may not be used in food categories for which no usage data have been provided. However, the Panel noted that given the information from the Mintel's Global New Products Database (GNPD), it may be assumed that acacia gum (E 414) is used in food categories for which no data have been provided by food industry.

The main food categories, in term of amount consumed, not taken into account were unflavoured fermented milk products, cheeses, breakfast cereals, foods for infants and young children (processed cereal-based foods and baby food, other foods for young children), snacks and some alcoholic beverages (cider and perry, spirit drinks, etc.). According to the Mintel GNPD (Appendix C), in the EU market, snacks and breakfast cereals are labelled with acacia gum (E 414), as well as few alcoholic drinks and nectars. Therefore, the Panel considered that if these uncertainties were confirmed, it would therefore result in an underestimation of the exposure.

The Panel noted that in Annex II of Regulation (EC) No 1333/2008, use levels of acacia gum (E 414) in food for infants under the age of 12 weeks are included in category 13.1.5.2. The Panel considered that these uses would require a specific risk assessment in line with the recommendations given by JECFA (1978) and the SCF (1998) and endorsed by the Panel (EFSA ANS

Panel, 2012). Therefore, the current re-evaluation of acacia gum (E 414) as a food additive is not considered to be applicable for infants under the age of 12 weeks and will be performed separately.

The Panel further noted that the exposure to acacia gum from its use according the Annex III (Part 1, 2, 3, 4 and 5) was not considered in the exposure assessment.

The Panel also noted that the refined exposure estimates are based on information provided on the reported level of use of acacia gum (E 414). If actual practice changes, this refined estimates may no longer be representative and should be updated.

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

- the safety assessment carried out by the Panel is limited to the use and use levels in 31 out of 76 food categories in which acacia gum (E 414) is authorised;
- an indicative high refined exposure assessment up to 719 mg/kg bw per day has been calculated in toddlers at the 95th percentile (non-brand loyal scenario) for the general population;
- an indicative high refined exposure assessment up to 626 mg/kg bw per day has been calculated in toddlers at the 95th percentile in the brand loyal scenario for the population consuming Foods for Special Medical Purposes (FSMPs);
- acacia gum is unlikely to be absorbed intact and is slightly fermented by intestinal microbiota;
- sufficient toxicity data were available;
- there is no concern with respect to the genotoxicity;
- no carcinogenic effects were reported in carcinogenicity studies in mice and rats at the doses up to 7,500 mg and 2,500 mg acacia gum/kg bw per day, respectively, the highest doses tested;
- oral daily intake of a large amount of acacia gum up to 30,000 mg acacia gum/person per day (approximately equivalent 430 mg acacia gum/kg bw per day) for up to 30 days was well tolerated in adults but some individuals experienced flatulence. A dose of 53,000 mg acacia gum/person per day (approximately equivalent 760 mg acacia gum/kg bw per day) induced mild flatulence, which was considered by the Panel as undesirable but not adverse,

the Panel concluded that there is no need for a numerical ADI for acacia gum (E 414), and that there is no safety concern at the refined exposure assessment for the reported uses of acacia gum (E 414) as a food additive.

Table of contents

1. Introduction

The present opinion document deals with the re-evaluation of acacia gum (E 414) when used as a food additive. Acacia gum (E 414) is an authorised food additive in the European Union (EU) according to Annex II and Annex III of Regulation (EC) No 1333/2008[1].

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

1.1.1. Background as provided by the European Commission

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

For this purpose, a programme for the re-evaluation of food additives that were already permitted in the European Union before 20 January 2009 has been set up under the Regulation (EU) No 257/2010[2]. This Regulation also foresees that food additives are re-evaluated whenever necessary in light of changing conditions of use and new scientific information. For efficiency and practical purposes, the re-evaluation should, as far as possible, be conducted by group of food additives according to the main functional class to which they belong.

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

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

1.1.2. Terms of Reference as provided by the European Commission

1.1.2.1. Re-evaluation of acacia gum (E 414) as a food additive

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

1.1.3. Interpretation of Terms of Reference

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

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

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

given by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1978, 1972) and by the SCF (1996; 1998). The Panel endorsed these recommendations.

In the current EU legislation (Annex III, part 5 section B of Regulation (EC) No 1333/2008), use of acacia gum in food for infants under the age of 12 weeks are authorised at the maximum level of 10 mg/kg carry over in final products included in categories 13.1. The Panel considers that these uses would require a specific risk assessment in line with the recommendations given by JECFA and the SCF and endorsed by the Panel in its current Guidance for submission for food additives evaluations (EFSA ANS Panel, 2012). Therefore, a risk assessment as for the general population is not considered to be applicable for infants under the age of 12 weeks and will be performed separately.

This re-evaluation refers exclusively to the uses of acacia gum (E 414) as a food additive in food, including food supplements and does not include a safety assessment of other uses of acacia gum.

1.2. Information on existing evaluations and authorisations

Acacia gum (E 414) is authorised as a food additive in the EU under Annex II of Regulation (EC) No 1333/2008 on food additives for use in foodstuffs. Specific purity criteria on acacia gum (E 414) have been defined in Commission Regulation (EU) No 231/2012.

In the EU, acacia gum has not been formally evaluated by the SCF and therefore no ADI has been allocated. However, it was accepted for use in weaning food (SCF, 1991). In 1999, the SCF considered 'that the use of acacia gum/gum arabic in coatings for nutrient preparations containing trace elements is acceptable provided carry-over levels in infant formulae, follow-on formulae or FSMP do not exceed 10 mg/kg' (SCF, 1999).

Acacia gum was evaluated by JECFA in 1982 and 1990 (JECFA, 1982, 1990). Based on the lack of adverse effects in the available toxicity studies, an ADI 'not specified' was allocated. It was stressed that the evaluation covered only acacia gum from *Acacia senegal* and closely related species; the specifications were amended in 1998 to cover also acacia gum from *Acacia seyal* (JECFA, 1998).

In 2010, the EFSA ANS Panel evaluated the use of gum acacia modified with octenyl succinic anhydride as a food additive (EFSA ANS Panel, 2010). Based on the results of the available studies, the information on gum acacia itself and on octenyl succinic anhydride modified starches, the Panel considered the use of octenyl succinic anhydride modified gum acacia as an emulsifier in foods at the proposed uses and use levels of no safety concern.

In 2010, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) prepared a scientific opinion on the substantiation of health claims related to acacia gum (EFSA NDA Panel, 2010). No cause and effect relationships could be established between the consumption of acacia gum and the reduction of post-prandial glycaemic responses or long-term maintenance of normal blood glucose concentrations.

Acacia gum is one of the food additives that composed jelly mini-cups which were suspended in 2004 by the European Commission to be placed on the market and import (Commission Decision 2004/37/EC), following the measures taken and information provided by different Member States. Jelly mini-cups are defined as 'jelly confectionery of a firm consistence, contained in semi rigid mini-cups or mini-capsules, intended to be ingested in a single bite by exerting pressure on the mini-cups or mini-capsule to project the confectionery into the mouth'.

In 2004, the EFSA Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) prepared a scientific opinion on a request from the European Commission related to the use of certain food additives derived from seaweed or non-seaweed origin, including acacia gum (E 414) in jelly mini-cups (EFSA AFC Panel, 2004). The AFC Panel concluded that any of these gel-forming additives or of any other type that gave rise to a confectionery product of a similar size, with similar physical and/or physicochemical properties and that could be ingested in the same way as the jelly mini-cups, would give rise to a risk for choking (EFSA AFC Panel, 2004). The use of these additives in jelly mini-cups is not authorised in the EU. The use of these additives in jelly mini-cups is not authorised in the EU.[3]

Acacia gum has also been reviewed by the Nordic Council of Ministers (TemaNord, 2002), who concluded that although the existing data do not point to any toxicological concern, the aspect of allergy/intolerance should be included in a future evaluation as well as the problem of marketing gums originating from acacia species not included in their evaluation.

[3] Annex II to Regulation (EC) No 1333/2008.

There is a monograph on acacia gum (named acacia) in the European Pharmacopoeia (2015). In this document, acacia gum is defined, and its solubility, identification and tests are indicated.

2. Data and methodologies

2.1. Data

The ANS Panel ANS was not provided with a newly submitted dossier. EFSA launched public calls for data,[4,5] to collect information from interested parties and, if relevant, contacted risk assessment bodies.

The Panel based its assessment on information submitted to EFSA following the public calls for data, information from previous evaluations and additional available literature up to 8 February 2017. Attempts were made at retrieving relevant original study reports on which previous evaluations or reviews were based; however, not always these were these available to the Panel.

The EFSA Comprehensive European Food Consumption Database (Comprehensive Database[6]) was used to estimate the dietary exposure.

The Mintel's Global New Products Database (GNPD) is an online resource listing food products and compulsory ingredient information that should be included in labelling. This database was used to verify the use of acacia gum (E 414) in food products.

2.2. Methodologies

This opinion was formulated following the principles described in the EFSA Guidance on transparency with regard to scientific aspects of risk assessment (EFSA Scientific Committee, 2009) and following the relevant existing guidance documents from the EFSA Scientific Committee.

The ANS Panel assessed the safety of acacia gum (E 414) as a food additive in line with the principles laid down in Regulation (EU) 257/2010 and in the relevant guidance documents: Guidance on submission for food additive evaluations by the Scientific Committee on Food (SCF, 2001) and taking into consideration the Guidance for submission for food additive evaluations in 2012 (EFSA ANS Panel, 2012).

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

Dietary exposure to acacia gum (E 414) from its use as a food additive was estimated combining food consumption data available within the EFSA Comprehensive European Food Consumption Database with the maximum levels according to Annex II to Regulation (EC) No 1333/2008[7] and/or reported use levels and analytical data submitted to EFSA following a call for data. Different scenarios were used to calculate exposure (see Section 3.3.1). Uncertainties on the exposure assessment were identified and discussed.

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

[4] Call for scientific data on food additives permitted in the EU and belonging to the functional classes of emulsifiers, stabilisers and gelling agents. Published: 22 November 2009. Available from: http://www.efsa.europa.eu/en/dataclosed/call/ans091123

[5] Call for technical data on certain thickening agents permitted as food additives in the EU – Extended Deadline: 31 December 2015. Available online: http://www.efsa.europa.eu/it/data/call/141219

[6] Available online: http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm

[7] Commission 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.

3. Assessment

3.1. Technical data

3.1.1. Identity of the substance

Acacia gum is a dried exudation obtained from the stems and branches of natural strains of *A. senegal* (L.) Willdenow or closely related species of *Acacia* (family Leguminosae) (Commission Regulation (EU) No 231/2012[8]; JECFA, 2006). Acacia gum (E 414) has the CAS Registry Number 9000-01-5 and the EINECS number 232-519-5.

Several works on the chemical and physicochemical characterisation of acacia gum are available. The polysaccharidic fractions of acacia gum consist of monomeric units being D-galactose, L-arabinose, L-rhamnose, D-glucuronic acid and 4-*O*-methyl-D-glucuronic acid (Randall et al., 1988; Fenyo and Vandevelde, 1990; Islam et al., 1997; Idris et al., 1998; Goodrum et al., 2000; Bracher et al., 2005; Dror et al., 2006; Mahendran et al., 2008; Sanchez et al., 2008; Renard et al., 2012, 2014; Nie et al., 2013). An interested party (Documentation provided to EFSA, n.5) provided the following concentration range of neutral sugars and uronic acids for the two commercial samples of acacia gum analysed in duplicate: galactose (32.5–35.0 molar%), arabinose (31.7–53.1 molar%), rhamnose (2.7–16.3 molar%), glucuronic acid (5.3–14.0 molar%) and 4-*O*-methyl-glucuronic acid (0.8–5.2 molar%).

Carbohydrate analysis has indicated that the components of this gum from the different sources, corresponding to three UV absorbance peaks, all have a highly branched structure consisting of a β-1,3-linked D-galactose core with extensive branching through 3- and 6-linked galactose and 3-linked arabinose. The main component is the arabinogalactan (AG) fraction that represents around 90% of the gum, containing less than 1% protein and with a molecular weight ~ 2.5×10^6 g/mol. The study of Sanchez et al. (2008) has shown that after purification, this fraction has a disk-like structure with a diameter of ~ 20 nm and a thickness below 2 nm. The second major component (~ 10 wt% of the total) consists of a higher molecular weight (~ 1–2 × 10^6 g/mol) arabinogalactan–protein (AGP) fraction and contains ~ 10% protein. Recent studies on the AGP fraction have been carried out (Mahendran et al., 2008; Renard et al., 2012); the smallest component (~ 1% of the total) consists of a glycoprotein (GP) fraction with the highest protein content (~ 50 wt%). A recent study has identified that in solution, the structure of the GP fraction is a mixture of spheroidal monomers and more anisotropic oligomers (Renard et al., 2014).

From this data, the Panel noted that nanosized particles (with one or more dimensions below 100 nm) could be present in the dry powder of acacia gum when used as a food additive.

The proteinaceous component of the first two fractions had similar amino acid distributions (hydroxyproline and serine the most abundant), while the amino acid composition of the GP fraction is different, with aspartic acid being the most abundant (Renard et al., 2006).

The gums from *A. senegal* and *A. seyal* have the same amino acid compositions and the same sugar residues, although the content of some of the sugar residues varies and the average molecular mass of the gum from *A. senegal* is higher than from that of *A. seyal* (Williams and Phillips, 2009).

As regards the protein content, the Panel noted that the protein content of hydrolysates of samples derived from *A. senegal* trees ranged from ca 1.5% to 3%, dependent on the area of production (Anderson et al., 1985). For various gums of the *Acacia* species, a slightly broader range of protein content (0.13–10.4%) has been reported by Phillips et al. (2008). According to documentation provided to EFSA (n.5), the content of proteins were in a range of 0.99–2.70% as determined in three samples of commercial acacia gum analysed in duplicate using the Kjeldahl method.

According to the EC specifications (Commission Regulation (EU) No 231/2012), the molecular weight of acacia gum is approximately 3.5 x 10^5 g/mol.

Acacia gum has for synonyms arabic gum, gum arabic, gum acacia, acacia, Senegal gum and Indian gum, among others.

Regarding the term gum arabic, the Panel noted that although most internationally traded gum arabic comes from *A. senegal*, the term 'gum arabic' does not indicate a particular botanical source. In a few cases, so-called 'gum arabic' may not even have been collected from *Acacia* species, but may originate from *Combretum*, *Albizia* or some other genus (FAO, 1999).

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

Unground acacia gum occurs as white or yellowish-white spheroidal tears of varying sizes or as angular fragments and is sometimes mixed with darker fragments. It is also available in the form of white to yellowish-white flakes, granules, powder or spray-dried material. One gram dissolves in 2 mL of cold water forming a solution, which flows readily. It is insoluble in ethanol (Commission Regulation (EU) No 231/2012). Solutions of food grades of acacia gum are practically odourless, colourless and tasteless (Klose and Glicksman, 1990).

3.1.2. Specifications

The specifications for acacia gum (E 414) as defined in the Commission Regulation (EU) No 231/2012 and by JECFA (2006) are listed in Table 1.

Table 1: Specifications for acacia gum (E 414) according to Commission Regulation (EU) No 231/2012 and JECFA (2006)

	Commission Regulation (EU) No 231/2012	**JECFA (2006)**
Definition	Acacia gum is a dried exudation obtained from the stems and branches of natural strains of *Acacia senegal* (L.) Willdenow or closely related species of *Acacia* (family Leguminosae). It consists mainly of high molecular weight polysaccharides and their calcium, magnesium and potassium salts, which on hydrolysis yield arabinose, galactose, rhamnose and glucuronic acid	Gum arabic is a dried exudate obtained from the stems and branches of *Acacia senegal* (L.) Willdenow or *Acacia seyal* (fam. Leguminosae). Gum arabic consists mainly of high-molecular weight polysaccharides and their calcium, magnesium and potassium salts, which on hydrolysis yield arabinose, galactose, rhamnose and glucuronic acid. Items of commerce may contain extraneous materials such as sand and pieces of bark, which must be removed before use in food
Molecular weight	Approximately 350,000	
Assay	–	–
Description	Unground acacia gum occurs as white or yellowish-white spheroidal tears of varying sizes or as angular fragments and is sometimes mixed with darker fragments. It is also available in the form of white to yellowish-white flakes, granules, powder or spray-dried material	Gum arabic (*A. senegal*) is a pale white to orange-brown solid, which breaks with a glassy fracture. The best grades are in the form of whole, spheroidal tears of varying size with a matt surface texture. When ground, the pieces are paler and have a glassy appearance. Gum arabic (*A. seyal*) is more brittle than the hard tears of gum arabic (*A. senegal*) Gum arabic is also available commercially in the form of white to yellowish-white flakes, granules, powder, roller dried or spray-dried material. An aqueous solution of 1 g in 2 mL flows readily and is acid to litmus
Identification		
Solubility	One gram dissolves in 2 mL of cold water forming a solution which flows readily and is acid to litmus, insoluble in ethanol	One gram dissolves in 2 mL of water; insoluble in ethanol
Gum constituents	–	Proceed as directed under Gum Constituents Identification (FNP 5) using the following as reference standards: arabinose, galactose, mannose, rhamnose, galacturonic acid, glucuronic acid and xylose. Arabinose, galactose, rhamnose and glucuronic acid should be present. Additional spots corresponding to mannose, xylose and galacturonic acid should be absent.

	Commission Regulation (EU) No 231/2012	JECFA (2006)
Optical rotation	–	Gum from *A. senegal*: aqueous solutions are levorotatory. Gum from *A. seyal*: aqueous solutions are dextrorotatory. Test a solution of 10 g of sample (dry basis) in 100 mL of water (if necessary, previously filtered through a No. 42 paper or a 0.8 μm Millipore filter) using a 200-mm tube
Purity		
Loss on drying	Not more than 17% (105°C, 5 h) for granular and not more than 10% (105°C, 4 h) for spray-dried material	Not more than 15% (105°, 5 h) for granular and not more than 10% (105°, 4 h) for spray-dried material
Total ash	Not more than 4%	Not more than 4%
Acid insoluble ash	Not more than 0.5%	Not more than 0.5%
Acid insoluble matter	Not more than 1%	Not more than 1%
Starch or dextrin	Boil a 1 in 50 solution of the gum and cool. To 5 mL add 1 drop of iodine solution. No bluish or reddish colours are produced	Boil a 1 in 50 solution of the sample, cool and add a few drops of Iodine TS. No bluish or reddish colour should be produced
Tannin	To 10 mL of a 1 in 50 solution add about 0.1 mL of ferric chloride solution (9 g $FeCl_3 \cdot 6H_2O$ made up to 100 mL with water). No blackish colouration or blackish precipitate is formed	To 10 mL of a 1 in 50 solution of the sample, add about 0.1 mL of ferric chloride TS. No blackish colouration or blackish precipitate should be formed
Arsenic	Not more than 3 mg/kg	–
Lead	Not more than 2 mg/kg	Not more than 2 mg/kg Determine using an atomic absorption technique 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, 'Instrumental Methods'
Mercury	Not more than 1 mg/kg	–
Cadmium	Not more than 1 mg/kg	–
Hydrolysis products	Mannose, xylose and galacturonic acid are absent (determined by chromatography)	–
Microbiological criteria		
Salmonella spp.	Absent in 10 g	Negative per test
Escherichia coli	Absent in 5 g	Negative in 1 g

The Panel noted that the JECFA specifications contain additional identification tests for gum constituents and for optical rotation. The Panel also noted that the EC specifications do not limit the protein content. Reports (Anderson et al., 1985; Phillips, 2008) indicate that the protein content could be from 0.13% to 10.4%. According to Documentation provided to EFSA (n.5), contents of proteins were in a range from 0.99% to 2.70% as determined in three samples analysed in duplicate. The interested party proposes the inclusion of the limit for protein content of 3.5% in the EC specifications.

No data was provided from interested parties concerning the enzymatic activities in acacia gum.

In literature, it is reported that the protein part of acacia gum may contain oxidising enzymes, especially oxidases and peroxidases, which may interact with easily oxidisable substances and which may be inactivated by heating acacia gum at 100°C for a short time (Leung and Foster, 2003; Bracher et al., 2005; Martindale, 2016). Oxidation of amines and phenols (e.g. eugenol, tannins, thymol, vanillin) by peroxidase present in acacia gum may lead to formation of coloured compounds (Leung and Foster, 2003). According to Glicksman and Sand, the oxidising enzymes present in acacia gum, may destroy, for example, the active component of pharmaceutical or nutritional preparations as demonstrated for vitamin A in emulsions of cod liver oil stabilised by gum acacia (Glicksman and Sand, 1973). Further

investigations of oxidative enzymes in polysaccharide gums seem only to have been performed on polyphenol oxidase from acacia gum, for which two isoenzymes were isolated and characterised (Billaud et al., 1996).

The Panel noted publications recommending that the oxidases and peroxidases present in the gum should be destroyed by heating of acacia gum during the manufacturing process or before its use. The Panel further noted that limits and a test for residual enzymatic activities and for protein content may be required.

An interested party (Documentation provided to EFSA, n.5) provided results of analysis of five batches of acacia gum for side components expressed as: loss on drying (7.6–14.2%), total ash (2.9–3.6%) and acid-insoluble matter (0.3–< 0.5%). All obtained results comply with the EC specifications.

A literature research done by the Panel revealed a limited number of papers describing microbiological contamination of different polysaccharide thickening agents (Souw and Rehm, 1973, 1975a,b); Robbins and Ingledew, 1975) but none of the results presented, gave rise to a particular concern. According to microorganism analysis on four different batches of acacia gum provided (Documentation provided to EFSA, n.5), the total plate count was in the range of 60–34,000 cfu/g and yeast and mould in the range of < 10–8,800 cfu/g.

Because of both the botanical origin and the polysaccharidic nature of gums, they can be a substrate of microbiological contamination and of field and storage fungal development. The latter has been recently demonstrated by the mycotoxin contaminations of gums (Zhang et al., 2014). The Panel noted that the differences in the microbiological criteria for acacia gum between the specifications given by the EU Regulation and those given by JECFA are not decisive. The Panel also noted that the microbiological specifications for polysaccharidic thickening agents, such as gums, should be harmonised and that for acacia gum criteria for the absence of *Salmonella* spp. and *Escherichia coli*, for total aerobic microbial count (TAMC) and total combined yeasts and moulds count (TYMC) should be included into the EU specifications as it is the case for other polysaccharidic thickening agents (e.g. alginic acids and its salts (E 400–E 404), agar (E 406), carrageenan (E 407), processed eucheuma sea weed (E 407a), xanthan gum (E 415), gellan gum (E 418)).

In view of the botanical origin of acacia gum, furthermore limitations of possible contamination with pesticides should be considered. According to industry (Documentation provided to EFSA, n.4), contents of pesticides are below the maximum levels as set in Regulation 1881/2006[9] on certain contaminants in foodstuff, and in Regulation 396/2005[10] on maximum residue levels of pesticides in or on food and feed of plant animal origin. Following a call for data, an interested party (Documentation provided to EFSA, n.5) indicated that no pesticides have been found in six different batches of acacia gum. However, in view of the use of acacia gum in baby and children food, the Panel considered particularly necessary to pay attention on the compliance of acacia gum (E 414) raw material to existing EU regulation on pesticides.

Information has been provided (Documentation provided to EFSA, n.5) on the content of toxic and other elements in five different batches of acacia gum: lead (< 0.02–0.036 mg/kg), mercury (< 0.005 mg/kg), cadmium (< 0.005–< 0.01 mg/kg), arsenic (< 0.005–< 0.1 mg/kg), aluminium (3.71–14.74 mg/kg), copper (1.1–1.59 mg/kg), iron (3.2–16.54 mg/kg) and zinc (< 0.5–< 1 mg/kg).

The Panel noted that the levels of lead, mercury, cadmium and arsenic in the five batches analysed, were all far below the levels as defined in the Commission Regulation (EU) No 231/2012 (Documentation provided to EFSA, n. 5).

The Panel noted that, according to the EC specifications for acacia gum (E 414), impurities of the toxic elements lead, mercury, cadmium and arsenic are accepted up to concentrations of 2, 1, 1 and 3 mg/kg, respectively. The Panel also noted that aluminium is not included in the specifications. Aluminium from all sources in food should not lead to an exceedance of a tolerable weekly intake (TWI) of 1 mg aluminium/kg bw (EFSA ANS Panel, 2008). Contamination at those levels could have a significant impact on the exposure to these metals, for which the intake is already close to the health-based guidance values established by EFSA (EFSA CONTAM Panel, 2009a,b, 2010, 2012).

The European Pharmacopeia (European Pharmacopoeia, 2015) includes specifications for acacia gum and spray-dried acacia gum. Spray-dried acacia gum is obtained from a solution of acacia gum

[9] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. OJ L 364, 20.12.2006, p. 5.

[10] Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. OJ L 70, 16.3.2005, p. 1.

and has the lower content of water (loss on drying not more than 10%) than the not spray-dried acacia gum (loss on drying not more than 15%).

3.1.3. Manufacturing process

Acacia gum is obtained from trees of the genus *Acacia*, subfamily Mimosoideae, family Leguminosae. The substance is the result of a bacterial or a fungal infection. It is exuded only by unhealthy trees; heat, poor nutrition and drought stimulate its production. The infection takes place through wounds in the tree which may be accidental or purposely made to stimulate gum production. The gum is exuded through these wounds in the bark in the form of tears, or drops which rapidly harden due to evaporation. Most of the acacia gum production is from wild trees, but some is from cultivated gardens which are tapped and collected on a systematic basis. After gathering, it is taken to central collecting stations where it is auctioned, graded by hand and dried (Klose and Glicksman, 1990).

As described in Thevenet, 2010:

> 'Raw gum from the same botanical origin is a blend of gum nodules with different mesh sizes, containing vegetable and mineral impurities and fluctuating bacteriological contamination. Using dry purifications steps, such as kibbling, sieving and pulverisation, the level of impurities can be slightly reduced but bacteriological contamination cannot be improved. Most of the time, raw gum does not meet the specifications for acacia gum. Consequently, the dry methods of purification have been substituted by purification in aqueous solution which is much more efficient. The gum is fully dissolved in water and all the impurities removed by a cascade of filtration steps giving levels of insoluble matter in the finished product as low as 0.02%. Bacterial contamination is reduced by treatment in a plate heat exchanger and the gum syrup is concentrated and dried, giving a level of microbial contamination in the powder not more than $5 \cdot 10^2$ germs per gram' (cfu/g).

> 'During solubilisation and purification, the thermal conditions are critical. Acacia gum contains proteins which are important for the emulsifying properties but sensitive to heat denaturation. Different processes are used for recovering purified, powdered acacia gum from the syrup. Roller drying is used to produce a gum in powder form with good hydration properties, but it has reduced emulsifying properties due to drastic thermal treatment during the drying step. Spray drying is also used which gives the gum good physical qualities and functional properties. Recently, spray drying has been improved by using a multi-stage spray drying process where fine particles of gum produced during drying are recycled at the top of the dryer. Agglomerated gum particles are obtained, keeping the entire properties of the raw gum, but containing no dust or particles below 75 μm and giving unique hydration and dissolution properties, without any lump formation up to the maximum level of solubility in water of 45–50%'.

The Panel noted publications recommending that during the manufacturing process the oxidases and peroxidases present in acacia gum should be inactivated by heating to prevent the possible oxidative degradation of components in preparations to which acacia gum is added (Glicksman and Sand, 1973; Ternes et al., 2007).

3.1.4. Methods of analysis in food

Acacia gum contains proteins and soluble dietary fibre and consists of arabinogalactans and other carbohydrate moieties. It is virtually impossible to quantify the exact concentration of the acacia gum after it has been added to food, since similar compounds are almost invariably present in foods and will interfere with the analysis. Even after treatment with protease, glucosidase, amylase, etc. (AOAC, 1998), there is no existing validated method to separate fibre mixtures into their individual fibre components (EFSA ANS Panel, 2010).

The problems in gum analysis are mainly arising due to diversity of structures and frequent use of blends. Most commonly used procedures for determining the amount of hydrocolloid in food involve the use of colorimetric methods or hydrolysis and determination of the monosaccharide composition; the latter may be carried out using high-performance liquid chromatography (HPLC). Methods that depend on interaction of hydrocolloids with plant lectins or antibodies have also been described (O'Donnell and Baird, 1993). A further method for the analysis of monosaccharides (galactose, glucose, arabinose, xylose and rhamnose) by thin-layer chromatography (TLC) is described in European Pharmacopoeia (2015).

Pazur and Li (2004) developed a technique for the identification of acacia gum in food using antibodies, isolated from the serum of rabbits immunised with this gum. Agar diffusion was performed with several foods (ice cream, soup, candy, salad dressing and cottage cheese) and antibody combinations. This method is highly specific for acacia gum.

3.1.5. Stability of the substance, and reaction and fate in food

Limited information on reaction and fate of acacia gum in foods is available.

Acacia gum is stable in acid conditions. Although the gum has excellent heat stability, prolonged storage of acacia gum solutions at high temperatures can result in the loss of some of the functional properties (Ullmann's Encyclopedia of Industrial Chemistry, 2012). Acacia gum contains proteins which are important for the emulsifying properties but are denaturated by heat (Thevenet, 2010).

The viscosity of acacia gum solutions decreases on ageing due to bacterial action and consequent depolymerisation. Reduction in viscosity has also been reported on prolonged exposure of solutions of the gum to ultrasonic vibration or ultraviolet irradiation. This was attributed to depolymerisation, and could be due to glycosidic fission as well as to disruption of physical aggregates (Williams et al., 2006).

3.2. Authorised uses and use levels

Maximum levels of acacia gum (E 414) have been defined in Annex II to Regulation (EC) No 1333/2008[7] on food additives, as amended. In this document, these levels are named maximum permitted levels (MPLs).

Currently, acacia gum (E 414) is an authorised food additive in the EU at *quantum satis* (QS) in all food categories listed in Table 2 apart from food category 13.1 Foods for infants and young children. Acacia gum (E 414) is included in the Group I of food additives authorised at QS.

Table 2 summarises foods that are permitted to contain acacia gum (E 414) and the corresponding MPLs as set by Annex II to Regulation (EC) No 1333/2008.

Table 2: MPLs of acacia gum (E 414) in foods according to the Annex II to Regulation (EC) No 1333/2008

Food category number	Food category name	Restrictions/exceptions	E-number/ group	MPL (mg/L or mg/kg as appropriate)
01.3	Unflavoured fermented milk products, heat-treated after fermentation		Group I	QS
01.4	Flavoured fermented milk products including heat-treated products		Group I	QS
01.6.3	Other creams		Group I	QS
01.7.1	Unripened cheese excluding products falling in category 16	Except mozzarella	Group I	QS
01.7.5	Processed cheese		Group I	QS
01.7.6	Cheese products (excluding products falling in category 16)		Group I	QS
01.8	Dairy analogues, including beverage whiteners		Group I	QS
02.2.2	Other fat and oil emulsions including spreads as defined by Council Regulation (EC) No 1234/2007 and liquid emulsions		Group I	QS
02.3	Vegetable oil pan spray		Group I	QS
03	Edible ices		Group I	QS
04.2.1	Dried fruit and vegetables		Group I	QS
04.2.2	Fruit and vegetables in vinegar, oil, or brine		Group I	QS
04.2.4.1	Fruit and vegetable preparations excluding compote		Group I	QS

Food category number	Food category name	Restrictions/exceptions	E-number/ group	MPL (mg/L or mg/kg as appropriate)
04.2.5.4	Nut butters and nut spreads		Group I	QS
04.2.6	Processed potato products		Group I	QS
05.1	Cocoa and Chocolate products as covered by Directive 2000/36/EC	As glazing agent only	E 414	QS
05.2	Other confectionery including breath refreshening microsweets	E 414 may not be used in jelly mini-cups, defined, for the purpose of this Regulation, as jelly confectionery of a firm consistence, contained in semi rigid mini-cups or mini-capsules, intended to be ingested in a single bite by exerting pressure on the mini-cups or mini-capsule to project the confectionery into the mouth	Group I	QS
05.3	Chewing gum		Group I	QS
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4		Group I	QS
06.2.2	Starches		Group I	QS
06.3	Breakfast cereals		Group I	QS
06.4.2	Dry pasta	Only gluten-free and/or pasta intended for hypoproteic diets in accordance with Directive 2009/39/EC	Group I	QS
06.4.4	Potato Gnocchi	Except fresh refrigerated potato gnocchi	Group I	QS
06.4.5	Fillings of stuffed pasta (ravioli and similar)		Group I	QS
06.5	Noodles		Group I	QS
06.6	Batters		Group I	QS
06.7	Precooked or processed cereals		Group I	QS
07.1.	Bread and rolls	Except products in 7.1.1 and 7.1.2	Group I	QS
07.2	Fine bakery wares		Group I	QS
08.3.1	Non-heat-treated meat products		Group I	QS
08.3.2	Heat-treated meat products	Except *foie gras, foie gras entier, blocs de foie gras, Libamáj, libamáj egészben, libamáj tömbben*	Group I	QS
08.3.3	Casings and coatings and decorations for meat		Group I	QS
09.2	Processed fish and fishery products including molluscs and crustaceans		Group I	QS
09.3	Fish roe	Only processed fish roe	Group I	QS
10.2	Processed eggs and egg products		Group I	QS
11.2	Other sugars and syrups		Group I	QS
11.4.1	Table Top Sweeteners in liquid form		E 414	QS
11.4.2	Table Top Sweeteners in powder form		E 414	QS

Food category number	Food category name	Restrictions/exceptions	E-number/ group	MPL (mg/L or mg/kg as appropriate)
11.4.3	Table Top Sweeteners in tablets		E 414	QS
12.1.2	Salt substitutes		Group I	QS
12.2.2	Seasonings and condiments		Group I	QS
12.3	Vinegars		Group I	QS
12.4	Mustard		Group I	QS
12.5	Soups and broths		Group I	QS
12.6	Sauces		Group I	QS
12.7	Salads and savoury based sandwich spreads		Group I	QS
12.8	Yeast and yeast products		Group I	QS
12.9	Protein products, excluding products covered in category 1.8		Group I	QS
13.1.3	Processed cereal-based foods and baby foods for infants and young children as defined by Directive 2006/125/EC	Only processed cereal based foods and baby foods[(b)]	E 414	10,000
13.1.3	Processed cereal-based foods and baby foods for infants and young children as defined by Directive 2006/125/EC	Only gluten-free cereal-based foods[(b)]	E 414	20,000
13.1.4	Other foods for young children	[(b)]	E 414	10,000
13.1.5.2	Dietary foods for babies and young children for special medical purposes as defined in Directive 1999/21/EC	Only processed cereal based foods and baby foods[(b)]	E 414	10,000
13.1.5.2	Dietary foods for babies and young children for special medical purposes as defined in Directive 1999/21/EC	Only gluten-free cereal-based foods[(b)]	E 414	20,000
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)		Group I	QS
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)		Group I	QS
13.4	Foods suitable for people intolerant to gluten as defined by Regulation (EC) No 41/2009	Including dry pasta	Group I	QS
14.1.2	Fruit juices as defined by Directive 2001/112/EC and vegetable juices	Only vegetable juices	Group I	QS
14.1.3	Fruit nectars as defined by Directive 2001/112/EC and vegetable nectars and similar products	Only vegetable nectars	Group I	QS
14.1.4	Flavoured drinks		Group I	QS
14.1.5.2	Other	Excluding unflavoured leaf tea; including flavoured instant coffee	Group I	QS
14.2.1	Beer and malt beverages		E 414	QS
14.2.3	Cider and perry		Group I	QS
14.2.4	Fruit wine and made wine		Group I	QS
14.2.5	Mead		Group I	QS

Food category number	Food category name	Restrictions/exceptions	E-number/ group	MPL (mg/L or mg/kg as appropriate)
14.2.6	Spirit drinks as defined in Regulation (EC) No 110/2008	Except whisky or whiskey	Group I	QS
14.2.7.1	Aromatised wines		Group I	QS
14.2.7.2	Aromatised wine-based drinks		Group I	QS
14.2.7.3	Aromatised wine-product cocktails		Group I	QS
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15% of alcohol		Group I	QS
15.1	Potato-, cereal-, flour- or starch-based snacks		Group I	QS
15.2	Processed nuts		Group I	QS
16	Desserts excluding products covered in category 1, 3 and 4		Group I	QS
17.1[(a)]	Food supplements supplied in a solid form including capsules and tablets and similar forms, excluding chewable forms		Group I	QS
17.2[(a)]	Food supplements supplied in a liquid form		Group I	QS
17.3[(a)]	Food supplements supplied in a syrup-type or chewable form		Group I	QS
18	Processed foods not covered by categories 1–17, excluding foods for infants and young children		Group I	QS

MPL: Maximum permitted level; QS: *quantum satis*.
(a): FCS 17 refers to food supplements as defined in Directive 2002/46/EC of the European Parliament and of the Council excluding food supplements for infants and young children.
(b): E 410, E 412, E 414, E 415 and E 440 are authorised individually or in combination.

According to Annex III, Part 1 of Regulation (EC) No 1333/2008, acacia gum (E 414) is also authorised in all food additives as a carrier at QS.

According to Annex III, Part 2 of Regulation (EC) No 1333/2008, acacia gum (E 414) is also authorised in all food additives other than carriers in food additives at QS.

According to Annex III, Part 3 of Regulation (EC) No 1333/2008, acacia gum (E 414) is also authorised as a food additive in food enzymes with a maximum level in the products (beverages or not) at QS.

In addition, according to Annex III, Part 4 of Regulation (EC) No 1333/2008, acacia gum (E 414) is authorised in food additives including carriers in all food flavourings at QS.

Finally, according to Annex III, Part 5, Section A and B of Regulation (EC) No 1333/2008, acacia gum (E 414) is also authorised at QS in all nutrients, as well as in all nutrients intended to be used in foods for infants and young children listed in Point 13.1 of Part E of Annex II, at the maximum level of 150,000 mg/kg in the nutrient preparation and 10 mg/kg carry-over in final products.

3.3. Exposure data

3.3.1. Reported use levels or data on analytical levels of acacia gum (E 414)

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

In the framework of Regulation (EC) No 1333/2008 on food additives and of Commission Regulation (EU) No 257/2010 regarding the re-evaluation of approved food additives, EFSA issued public calls,[11,12] for occurrence data (usage level and/or concentration data) on acacia gum (E 414). In response to these public calls, updated information on the actual use levels of acacia gum (E 414) in foods was made available to EFSA by industry (food industry and gum manufacturers). No analytical data on the concentration of acacia gum (E 414) in foods were made available by the Member States.

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

Updated information on the actual use levels (n = 287) of acacia gum (E 414) in foods was made available to EFSA by Association for international Promotion of Gums (AIPG), Associazione Industriali delle Carni e dei Salumi, Association of the European Self-Medication Industry (AESGP), DOMACO Dr. med. Aufdermaur AG, FoodDrinkEurope (FDE), Frutarom Industries Ltd, interested party providing Documentation n.11, F. Hunziker + Co AG, A.H. Meyer & Cie AG, International Chewing Gum Association (ICGA), Nathura, Specialised Nutrition Europe (SNE), CHEPLAPHARM Arzneimittel GmbH, Rudolf Wild GmbH & Co. KG and Stollwerck.

The Panel noted that some data providers (e.g. Association for international Promotion of Gums, Rudolf Wild GmbH & Co) are not food industry using gums in their food products but food additive producers. Usage levels reported by food additive producers should not be considered at the same level as those provided by food industry. Food additive producers might recommend usage levels to the food industry but the final levels might, ultimately, be different, unless food additive producers confirm that these levels are used by food industry. In all other cases, data from food additive producers will only be used in the MPL scenario in case of QS authorisation when no data are available from food industry in order to have the most complete exposure estimates.

Appendix A provides data on the use levels of acacia gum (E 414) in foods as reported by industry.

3.3.2. Summarised data extracted from the Mintel GNPD database

The Mintel's GNPD is an online database which monitors product introductions in consumer packaged goods markets worldwide. It contains information of over 2 million food and beverage products of which more than 900,000 are or have been available on the European food market. Mintel started covering EU's food markets in 1996, currently having 20 out of its 28 member countries and Norway presented in the GNPD.[13]

For the purpose of this Scientific Opinion, GNPD[14] was used for checking the labelling of products containing acacia gum (E 414) within the EU's food products as GNPD shows the compulsory ingredient information presented in the labelling of products.

In the 20 EU countries, acacia gum (E 414) is labelled on almost 13,000 foods and drinks with over 8,200 of them published between 2011 and 2016.

Appendix B presents the percentage of the food products labelled with acacia gum (E 414) between 2011 and 2016, out of the total number of food products per food subcategories according to the Mintel food classification.

3.3.3. Food consumption data used for exposure assessment

3.3.3.1. EFSA Comprehensive European Food Consumption Database

Since 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) has been populated with national data on food consumption at a detailed level. Competent authorities in the European countries provide EFSA with data on the level of food consumption by the individual consumer from the most recent national dietary survey in their country (cf. Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011a). New consumption surveys recently[15] added in the Comprehensive database were also taken into account in this assessment.[16]

[11] http://www.efsa.europa.eu/sites/default/files/consultation/ans091123.pdf
[12] http://www.efsa.europa.eu/sites/default/files/consultation/140310.pdf
[13] Missing Bulgaria, Cyprus, Estonia, Latvia, Lithuania, Luxembourg, Malta and Slovenia.
[14] http://www.gnpd.com/sinatra/home/ accessed on 21/11/2016.
[15] Available online: http://www.efsa.europa.eu/en/press/news/150428.htm
[16] Available online: http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm

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

Food consumption data from the following population groups: infants, toddlers, children, adolescents, adults and the elderly were used for the exposure assessment. For the present assessment, food consumption data were available from 33 different dietary surveys carried out in 19 European countries (Table 3).

Table 3: Population groups considered for the exposure estimates of acacia gum (E 414)

Population	Age range	Countries with food consumption surveys covering more than 1 day
Infants	From more than 12 weeks up to and including 11 months of age	Bulgaria, Denmark, Finland, Germany, Italy, UK
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Denmark, Finland, Germany, Italy, Netherlands, Spain, UK
Children[(a)]	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Spain, Sweden, UK
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Italy, Latvia, Spain, Sweden, UK
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Romania, Spain, Sweden, UK
The elderly[(a)]	From 65 years of age and older	Austria, Belgium, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Romania, Sweden, UK

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

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

3.3.3.2. Food categories considered for the exposure assessment of acacia gum (E 414)

The food categories in which the use of acacia gum (E 414) is authorised were selected from the nomenclature of the EFSA Comprehensive Database (FoodEx classification system), at the most detailed level possible (up to FoodEx Level 4) (EFSA, 2011b).

Some food categories or their restrictions/exceptions are not referenced in the EFSA Comprehensive Database and could therefore not be taken into account in the present estimate. This was the case for 10 food categories and may have resulted in an underestimation of the exposure. The food categories which were not taken into account are described below (in ascending order of the FCS codes):

- 01.6.3 Other creams;
- 02.3 Vegetable oil pan spray;
- 06.4.4 Potato gnocchi;
- 06.6 Batters;
- 06.7 Precooked or processed cereals, only precooked cereals;
- 08.3.3 Casings and coatings and decorations for meat;
- 12.1.2 Salts substitutes;
- 13.1.3 Processed cereal-based foods and baby foods for infants and young children as defined by Commission Directive 2006/125/EC, only gluten-free cereal-based foods;
- 13.1.5.2 Dietary foods for babies and young children for special medical purposes as defined in Directive 1999/21/EC, only gluten-free cereal-based foods;

- 14.1.3 Fruit nectars as defined by Directive 2001/112/EC and vegetable nectars and similar products, only vegetable nectars.

For the following food categories, the restrictions/exceptions which apply to the use of acacia gum (E 414) could not be taken into account, and therefore, the whole food category was considered in the exposure assessment. This applies to five food categories and may have resulted in an overestimation of the exposure:

- 05.1 Cocoa and Chocolate products as covered by Directive 2000/36/EC, as glazing agent only;
- 05.2 Other confectionery including breath refreshening microsweets, may not be used in jelly mini-cups;
- 07.1 Bread and rolls, except products in 7.1.1 and 7.1.2;
- 08.3.2 Heat-treated meat products, except *foie gras, foie gras entier, blocs de foie gras, Libamáj, libamáj egészben, libamáj tömbben*;
- 09.3 Fish roe, only processed fish roe.

For the maximum level exposure assessment scenario, one added food category was not taken into account because no concentration data were provided to EFSA. For the refined scenario, 31 added food categories were not taken into account because only concentration data provided by food additive manufacturers were made available to EFSA.

For the remaining food categories, the refinements considering the restrictions/exceptions as set in Annex II to Regulation No 1333/2008 were applied. Overall, for the maximum level exposure scenario, 61 food categories were included, while for the refined scenarios, 31 food categories were included in the present exposure assessment to acacia gum (E 414) (Appendix B).

3.4. Exposure estimates

3.4.1. Exposure to acacia gum (E 414) from its use as a food additive

The Panel estimated chronic exposure to acacia gum (E 414) for the following population groups: infants; toddlers, children, adolescents, adults and the elderly. Dietary exposure to acacia gum (E 414) was calculated by multiplying acacia gum (E 414) concentrations for each food category (Appendix C) with their respective consumption amount per kilogram of body weight for each individual in the Comprehensive Database. The exposure per food category was subsequently added to derive an individual total exposure per day. These exposure estimates were averaged over the number of survey days, resulting in an individual average exposure per day for the survey period. Dietary surveys with only 1 day per subject were excluded as they are considered as not adequate to assess repeated exposure.

This was carried out for all individuals per survey and per population group, resulting in distributions of individual exposure per survey and population group (Table 3). On the basis of these distributions, the mean and 95th percentile of exposure were calculated per survey and per population group. The 95th percentile of exposure was only calculated for those population groups where the sample size was sufficiently large to allow this calculation (EFSA, 2011a). Therefore, in the present assessment, the 95th percentile of exposure for infants from Italy and for toddlers from Belgium, Italy and Spain were not included.

It should be noted that, in a dietary surveys from Finland, namely DIPP_2001_2009 (EFSA, 2011a), the consumption of grain-based products including bread and fine bakery products was coded at the level of their ingredients (flour), which resulted in a very low exposure to locust bean gum in all Finnish populations compared with the other studies. Therefore, this study was excluded from the assessment.

Exposure assessment to acacia gum (E 414) was carried out by the ANS Panel based on (1) maximum levels of data provided to EFSA (defined as the *maximum level exposure assessment scenario*) and (2) reported use levels (defined as the *refined exposure assessment scenario*) as provided by industry. These two scenarios are discussed in detail below.

These scenarios do not consider the consumption of food supplements (FC 17.1, FC 17.2 and FC 17.3) nor the consumption of foods for special medical purposes (FSMP) which are covered in additional refined exposure scenarios detailed below (*food supplements consumers only scenario and food for special medical purposes consumer only scenario*).

As acacia gum (E 414) is also authorised in the food category 13.1.5.2, a refined estimated exposure assessment scenario taking into account this food category was performed to estimate the exposure of infants and toddlers who may eat and drink these FSMP. This scenario does not consider the consumption of food supplements.

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

FSMP consumed in other population groups (children, adolescents, adults and the elderly) may be very diverse; they cannot be considered because of very limited information on consumption. Eating occasions belonging to the food categories 13.2, 13.3 and 13.4 were therefore reclassified under food categories in accordance to their main component.

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

Concerning the uses of acacia gum (E 414) as carriers, there might be food categories where acacia gum is used according to annex III and not to annex II. These food categories can only be addressed by analytical data or limits set in the Regulation No 1333/2008. According to Annex III, Part 5, Section B of Regulation (EC) No 1333/2008, acacia gum (E 414) is also authorised at QS in all nutrients intended to be used in foods for infants and young children listed in Point 13.1 of Part E of Annex II, at the maximum level of 150,000 mg/kg in the nutrient preparation and 10 mg/kg carry-over in final products. Therefore, this maximum level was taken into account in the MPL scenario. As a reported use levels was made available to EFSA for the FC 13.1.5.1, this food category was taken into in the refined exposure scenario.

3.4.1.1. Maximum level exposure assessment scenario

The regulatory maximum level exposure assessment scenario is based on the MPLs as set in Annex II to Regulation (EC) No 1333/2008. As acacia gum (E 414) is authorised according to QS in almost all food categories, a 'maximum level exposure assessment' scenario was estimated based on the maximum reported use levels provided by industry, as described in the EFSA Conceptual framework (EFSA ANS Panel, 2014).

The Panel considers the exposure estimates derived following this scenario as the most conservative as it is assumed that that the population group will be exposed to acacia gum (E 414) present in food at the maximum reported use levels over a longer period of time.

3.4.1.2. Refined exposure assessment scenario

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

Appendix C summarises the concentration levels of acacia gum (E 414) used in the refined exposure assessment scenario. Based on the available data set, the Panel calculated two refined exposure estimates based on different model populations:

- The brand-loyal consumer scenario: It was assumed that a consumer is exposed long-term to acacia gum (E 414) present at the maximum reported use for one food category. This exposure estimate is calculated as follows:
  - Combining food consumption with the maximum of the reported use levels for the main contributing food category at the individual level.
  - Using the mean of the typical reported use levels for the remaining food categories.
- The non-brand-loyal consumer scenario: It was assumed that a consumer is exposed long-term to acacia gum (E 414) present at the mean reported use in food. This exposure estimate is calculated using the mean of the typical reported use levels for all food categories.

As mentioned above, 2 specific scenarios were also performed:

- Food supplements consumers only scenario: This scenario was estimated as follows:
  - Consumers only of food supplements were assumed to be exposed to acacia gum (E 414) present at the maximum reported use level on a daily basis via consumption of food supplements. For the remaining food categories, the mean of the typical reported use levels was used.

As food category 17 does not consider food supplements for infants and toddlers as defined in the legislation, exposure to food additives from food supplements is not estimated for these two population groups.

- Food for special medical purposes consumers only scenario: This scenario was estimated as follows:
  - Consumers only of foods for special medical purposes were assumed to be exposed to acacia gum (E 414) present at the maximum reported use level on a daily basis via consumption of food category 13.1.5.2. For the remaining food categories, the mean of the typical reported use levels was used.

3.4.1.3. Dietary exposure to acacia gum (E 414)

Table 4 summarises the estimated exposure to acacia gum (E 414) from its use as a food additive in six population groups (Table 3) according to the different exposure scenarios (Section 3.4.1). Detailed results per population group and survey are presented in Appendix C.

Table 4: Summary of dietary exposure to acacia gum (E 414) from its use as a food additive in the maximum level exposure assessment scenario and in the refined exposure scenarios, in six population groups (minimum–maximum across the dietary surveys in mg/kg bw per day)

	Infants (12 weeks–11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Maximum level exposure assessment scenario						
Mean	242.1–880.9	309.8–1,398.0	314.5–1,056.0	196.2–671.0	87.8–350.9	69.2–278.4
95th percentile	705.5–2,952.2	1,108.08–2,767.2	824.4–1,994.0	458.1–1,433.1	221.6–811.4	150.0–657.1
Refined estimated exposure assessment scenario						
Brand-loyal scenario						
Mean	24.0–135.1	116.8–820.3	198.2–667.9	101.1–423.9	50.8–201.9	40.6–119.8
95th percentile	141.1–444.5	579.2–1,735.6	529.9–1,546.4	290.0–1,055.3	124.9–566.5	91.0–249.1
Non-brand-loyal scenario						
Mean	4.8–43.7	64.5–317.7	86.9–276.3	35.0–146.0	15.0–65.2	16.7–43.9
95th percentile	59.0–160.4	215.7–719.2	231.5–615.1	88.6–419.1	43.7–175.9	40.7–105.4

From the *regulatory maximum level exposure assessment scenario*, mean exposure to acacia gum (E 414) from its use as a food additive ranged from 69.2 mg/kg bw per day for the elderly to 1,398 mg/kg bw per day in toddlers. The 95th percentile of exposure to acacia gum (E 414) ranged from 150 mg/kg bw per day for the elderly to 2,952.2 mg/kg bw per day in infants.

From the *refined estimated exposure scenario*, in the *brand-loyal scenario*, mean exposure to acacia gum (E 414) from its use as a food additive ranged from 24 mg/kg bw per day in infants to 820.3 mg/kg bw per day in toddlers. The high exposure to acacia gum (E 414) ranged from 91 mg/kg bw per day for the elderly to 1,735.6 mg/kg bw per day in toddlers. In the *non-brand-loyal scenario*, mean exposure to acacia gum (E 414) from its use as a food additive ranged from 4.8 mg/kg bw per day in infants to 317.7 mg/kg bw per day in toddlers. The 95th percentile of exposure to acacia gum (E 414) ranged from 40.7 mg/kg bw per day for the elderly to 719.2 mg/kg bw per day in toddlers.

From the *refined estimated exposure scenario taking into account the foods for special medical purposes*, consumers only, mean exposure to acacia gum (E 414) from its use as a food additive ranged for infants between 22 and 117 mg/kg bw per day and between 69 and 378 mg/kg bw per day for toddlers. The 95th percentile of exposure to acacia gum (E 414) ranged for infants between 89 and 317 mg/kg bw per day and for toddlers between 175 and 626 mg/kg bw per day. The food categories contributing the most at the mean exposure level are foods for infants and young children for infants and confectionary, foods for infants and young children and fine bakery wares for toddlers.

From the *refined estimated exposure scenario taking into account the consumption of food supplements*, consumers only, among children, adolescents, adults and the elderly, mean exposure to acacia gum (E 414) from its use as a food additive ranged between 18 and 424 mg/kg bw per day. The 95th percentile of exposure ranged between 83 and 521 mg/kg bw per day.

3.4.1.4. Main food categories contributing to exposure to acacia gum (E 414) using the maximum level exposure assessment scenario

The main contributing food categories to the mean exposure estimates for infants in this scenario were foods for infants and young children (FCS 13.1) and unflavoured fermented milk products; for toddlers, they were unflavoured fermented milk products, breakfast cereals and confectionary; for children, they were confectionary, flavoured drinks and unflavoured fermented milk products; confectionary and flavoured drinks were also the main contributing food categories for adolescents and adults. For the elderly, the main contributing food categories were breakfast cereals, coffee, tea, herbal and fruit infusions and bread and rolls (see Table 5 for more details).

3.4.1.5. Main food categories contributing to exposure to acacia gum (E 414) using the refined exposure assessment scenario

In the *brand-loyal scenario*, the main contributing food categories were bread and rolls for infants, confectionary for toddlers, children, adolescents; flavoured drinks for adults and coffee, tea, herbal and fruit infusions for the elderly In the *non-brand-loyal scenario*, the main contributing food categories were fine bakery wares for infants, fine bakery wares and confectionary for toddlers, confectionary for children and adolescents and fine bakery wares for adults and the elderly (see Tables 6 and 7 for more details).

Tables 5, 6 and 7 can be found in the online version of this output ('Supporting information' section): https://doi.org/10.2903/j.efsa.2017.4741

3.4.1.6. Uncertainty analysis

Uncertainties in the exposure assessment of acacia gum (E 414) have been discussed above. In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2007), the following sources of uncertainties have been considered and summarised in Table 8.

Table 8: Qualitative evaluation of influence of uncertainties on the dietary exposure estimate

Sources of uncertainties	Direction[a]
Consumption data: different methodologies/representativeness/underreporting/misreporting/no portion size standard	+/–
Use of data from food consumption survey of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Correspondence of reported use levels to the food items in the EFSA Comprehensive Food Consumption Database: • uncertainties to which types of food the levels refer to • levels considered applicable for all items within the entire food category	 +/– +/–
Uncertainty in possible national differences in use levels of food categories	+/–
Concentration data: • levels considered applicable for all items within the entire food category, • not fully representative of foods on the EU market	 + +/–
Range from 7% to 96% of the amount (g of foods by body weight) of food consumed taken into account in the refined exposure assessment scenarios out of all authorised food (n = 31/76 food categories)	–
Food categories selected for the exposure assessment: • exclusion of food categories due to missing FoodEx linkage (n = 10/76 food categories)	–
Food categories selected for the exposure assessment: inclusion of food categories without considering the restriction/exception (n = 5/76 food categories)	+

Sources of uncertainties	Direction[a]
Maximum level exposure assessment scenario:	
• exposure calculations based on the maximum reported use levels (reported use from industries)	+
• food categories which may contain acacia gum (E 414) due to carry-over not considered	–
• food categories authorised at MPL according to Annex II to Regulation (EC) No 1333/2008	+
• data not available for certain food categories which were excluded from the exposure estimates (n = 1/76 food categories)	–
Refined exposure assessment scenarios:	
• food categories which may contain acacia gum (E 414) due to carry-over not considered	–
• exposure calculations based on the maximum or mean levels (reported use from industries)	+/–
• data not available for certain food categories which were excluded from the exposure estimates (n = 31/76 food categories)	–

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

Acacia gum (E 414) is authorised as a Group I food additive in 66 food categories and has a specific authorised uses in 10 other categories (Table 2). Since, the majority of food categories correspond to the general Group I food additives authorisation, acacia gum (E 414) may not necessarily be used in some of these food categories. This may explain why use levels reported by food industry for acacia gum (E 414) was not available for 31 food categories.

Furthermore, the Panel noted that information from the Mintel's GNPD (Appendix B) indicated that some of these 31 food categories were labelled with acacia gum (breakfast cereals, snacks, cheeses).

Overall, the Panel considered that the uncertainties identified would, in general, result in an overestimation of the exposure to acacia gum (E 414) as a food additive in European countries considered in the EFSA European database for the maximum level exposure scenario and for the refined scenario if it is considered that the food additive are not used in food categories for which no usage data have been provided.

However, the Panel noted that given the information from the Mintel's GNPD, it may be assumed that acacia gum (E 414) is used in food categories for which no data have been provided by food industry. If this was confirmed, it would therefore result in an underestimation of the exposure.

The Panel noted that food categories which may contain acacia gum (E 414) due to carry-over (Annex III, Part 1, 2, 3, 4 and 5 (sections A and B)) were not considered in the current exposure assessment.

Considering the exposure to acacia gum (E 414) for infants and young children eating FSMPs, the Panel considered that the uncertainties identified would, in general, result in an overestimation of the exposure in European countries considered in the EFSA European database for the brand-loyal refined scenario.

3.4.2. Exposure via other uses

Exposure to acacia gum due to the following uses were not considered in this opinion.

3.4.2.1. Acacia gum as an ingredient in food supplements and other foods

In literature, the use of acacia gum as 'soluble dietary fibre' in food is described (Cherbut et al., 2003).

3.4.2.2. Pharmaceutical uses

For acacia gum as an active ingredient, no authorised medicinal products exist within the EU (Documentation provided to EFSA, n.8).

Acacia gum is used in pharmaceutical products only as an excipient, e.g. as a suspending and emulsifying agent, as an adhesive and binder in tableting and demulcent syrups (Verbeken et al., 2003; Martindale, 2014).

3.5. Biological and Toxicological data

The biological properties of acacia gum (E 414) have been previously evaluated by JECFA in 1982 and 1990 (JECFA, 1982, 1990). The present opinion briefly reports the main studies evaluated in these reports. Additional information has been identified from literature (CIR, 2005) and from a new literature search. In most of the studies evaluated, the identity of the test material (acacia gum or gum arabic) was not specified.

The Panel noted that the toxicological database on acacia gum is mostly derived from its use as an emulsifying agent in studies of the toxicity of water-insoluble chemicals.

3.5.1. Absorption, distribution, metabolism and excretion

There is evidence that certain high molecular weight dietary polysaccharides, such as gums, could be partially broken down in the large intestine of man. In addition to intermediate metabolites, such as lactate, acrylate or fumarate, the main end products of this colonic anaerobic digestive process are short-chain fatty acids (SCFA), such as acetic, propionic and butyric acids, which are absorbed from the colon (Cummings and Englyst, 1987).

3.5.1.1. *In vitro* studies

A total of 188 strains from 11 species *Bacteroides* species found in the human colon were surveyed for their ability to ferment mucins and plant polysaccharides including gums (Salyers et al., 1977a). Many of the *Bacteroides* strains tested were able to ferment a variety of plant polysaccharides, including amylose, dextran, pectin and gums. The ability to utilise mucins and plant polysaccharides varied considerably among the *Bacteroides* species tested. By contrast to other gums (locust bean gum or gum tragacanth), none of the *Bacteroides* tested fermented acacia gum (origin, Meer Co).

A total of 154 strains from 22 species of *Bifidobacterium, Peptostreptococcus, Lactobacillus, Ruminococcus, Coprococcus, Eubacterium* and *Fusobacterium*, which are present in high concentrations in the human colon, were surveyed for their ability to ferment 21 different complex carbohydrates, including gums (Salyers et al., 1977b). Among them, acacia gum (origin, Meer Co) was fermented by some strains of *Bifidobacterium* species.

Adiotomre et al. (1990) investigated the effects of dietary fibres, including gums, on caecal fermentations by using fresh human microflora. Evolution of SCFAs and water-holding capacity after fermentation were also measured. Among other gums, acacia gum (E 414) yielded the largest amount of total SCFAs (74.0 vs 15.5 mmol/L for controls). The major SCFAs produced were acetic, propionic and butyric acids, with smaller amounts of isobutyric, valeric and isovaleric acids. By contrast, the amount of water held by 1 g of the fermented residue was low as compared to other fibres in case of acacia gum (2.05 vs 0.91 g/g for controls).

A total of 290 strains of 29 species of bifidobacteria of human and animal origin (mainly of faecal origin) were surveyed for their ability to ferment complex carbohydrates (Crociani et al., 1994). The substrates fermented by the largest number of species were D-galactosamine, D-glucosamine, amylose and amylopectin. Acacia gum (origin, Sigma Co) was shown to be mainly fermented by *Bifidobacterium longum* strains.

In another *in vitro* study, acacia gum (90.8% dry matter with 2.4% crude protein) was fermented using dog faeces as the source of inoculum (Sunvold et al., 1995a). Organic matter disappearance and SCFAs production was measured after 6, 12 or 24 h of incubation. Whatever the duration of incubation was, the organic matter disappearance, and acetate, propionate and butyrate productions were lower for acacia gum than for other gums. Identical conclusions were drawn from a similar study using the same substrates fermented by cat faecal microflora (Sunvold et al., 1995b).

Kishimoto et al. (2006) used enriched cultures of pig caecal bacteria to investigate the fermentation of a selected high molecular weight specific acacia gum (MW 1.77 x 10^6 g/mol, a specific gum arabic, namely *A. senegal* (L.) Willd. designated Acacia (sen) SUPER GUMTM EM2)). In this *in vitro* study, a *Prevotella ruminicola*-like bacterium was found as a predominant bacterium that is most likely to be responsible for fermentation of acacia gum to propionate.

3.5.1.2. *In vivo* studies

After fasting for 48 h, 20 young male rats (strain not specified, average weight 140 g) received orally 10 g of a mixture containing 34% acacia gum (white power, unspecified origin) in cocoa butter (Monke, 1941). Rats were killed 72 h later and their livers were removed and the glycogen levels

determined. The differences in liver glycogen levels between control and acacia gum-treated rats were not significant.

Groups of five rats (strain not specified) were pair-fed acacia gum (0.75 g/day in 5 g basal diet, (unspecified origin)). The digestibility of gum arabic was reported to be 71% (no further information was available) (in: Informatics Inc., 1972, referring to Booth et al., 1963).

Acacia gum (unspecified origin) was found to be highly digestible by guinea pigs (O'Dell et al., 1957) or by rats (Shue et al., 1962). However, the amounts of acacia gum in the diet or the exposure times were not specified. Therefore, these studies are of limited value for a final conclusion.

Later studies showed that acacia gum (unspecified origin) is partially digested by the rat (Booth et al., 1963). Weight gain and feed efficiency (gain/feed intake) was determined in six rats (strain and sex not specified, reported as albino rat, mean starting weight 37,500 mg, about 20 days old) fed 15% acacia gum for 62 days (equivalent[17] to 18,000 mg acacia gum/kg bw day). The feed efficiencies were identical in treated and control group but the rats given gum arabic had a mean weight gain of 224 g and the controls of 199 g, suggesting that gum arabic was utilised. Information regarding absorption is not reported in the publication.

The metabolism of acacia gum (conformed to the British Pharmacopoeia specification) was studied in male albino Wistar rats receiving acacia gum (form unspecified) incorporated into a high protein containing diet (Oxoid breeders) for 2 weeks (Ross et al., 1981, 1984). Animal numbers in treatment groups varied from 3 to 25 for the specific investigation. This diet was chosen for its high protein content in order to minimise the nutritional deficiency caused by the addition of large proportions of acacia gum (25,000, 50,000, 100,000 and 200,000 mg/kg diet, equivalent to 2,950, 5,900, 11,800 and 23,600 mg acacia gum/kg bw per day, respectively). An alternative method was to incorporate the acacia gum into a low-residue, nutritionally complete elemental diet up to 130,000 mg/kg (equivalent to 15,300 mg acacia gum/kg bw per day). It was observed, that acacia gum could be recovered from the small intestine, but not from the caecum, colon, rectum or faeces of the treated rats. By contrast, in rats where caecum was surgically removed with restoration of intestinal continuity, acacia gum was detected all along the gastrointestinal tract, from stomach to rectum and also in the faeces. The authors concluded that acacia gum is not significantly degraded in the upper gastrointestinal tract, but is rapidly decomposed by bacterial activity within the caecum, associated with increased breath methane excretion, increased volatile fatty acid concentrations and changes in the proportions of various volatile fatty acids in the faeces.

3.5.1.3. Human study

Five healthy male volunteers (33–55 years old) ingested daily doses of 25,000 mg acacia gum (approx. 350 mg acacia gum/kg bw per day, as a solution in 7% dextrose, conformed to the British Pharmacopoeia specification) for 21 days (Ross et al., 1983). Acacia gum was not recovered in faeces. According to the authors, the gum must have been digested during passage through the human colon. The dose was well tolerated. According to the authors, the absence of precipitable acacia gum in the faeces of the subjects and the marked increases in breath hydrogen production would indicate that the molecule is degraded during its passage through the human colon and that microflora is responsible for this.

Overall, the *in vitro* degradation and the *in vivo* digestibility of acacia gum have been investigated in animal and human models. These studies demonstrated that acacia gum would not be absorbed intact and would not be metabolised by enzymes present in the gastrointestinal tract. However, it would be partially fermented during its passage through the large intestine by the action of the intestinal tract microflora. The rate of hydrolysis in the gastrointestinal tract in humans is unknown, but it is expected that the limited extent of hydrolysis of acacia gum would lead to the production of its fermentation products such as SCFAs. Based on the available knowledge on the role of SCFA as end products of the fermentation of dietary fibres by the anaerobic intestinal microflora (Den Besten et al., 2013; Topping and Clifton, 2001), the Panel considered that their potential formation as fermentation products from acacia gum does not raise a safety concern.

3.5.2. Acute toxicity

The acute oral toxicity of acacia gum (unspecified origin) was tested in mouse, rat, hamster and rabbit. The substance was given once by gavage as a 25% suspension in corn oil (FDRL, 1972a). The

[17] EFSA guidance on selected default values. EFSA Journal 2012;10(3):2579, 32 pp.

LD_{50} values were higher than 16,000, 18,000 and 16,000 mg/kg bw in mouse, rat and hamster, respectively. In rabbits, the LD_{50} was 8,000 mg/kg bw.

In another acute oral toxicity study in rabbits (weights and strain not stated), a LD_{50} of 80 000 mg/kg bw was reported for acacia gum (CIR, 2005).

3.5.3. Short-term and subchronic toxicity

Acacia gum tested in the following studies was not fully characterised.

Groups of six male Sprague–Dawley rats (3 weeks old) were fed a diet containing 50,000 mg acacia gum/kg diet (equivalent to 5,900 mg acacia gum/kg bw per day) for 4 weeks (Mallett et al., 1984). Treatment had no effect on body weight, but the weight of the caecal wall and of the caecal contents was significantly increased. In addition, concentration of caecal ammonia was increased. There was also a significant increase in the activity of bacterial enzymes such as azo reductase, nitroreductase and nitrate reductase as compared to controls.

In a study of Anderson et al. (1984), groups of three male Wistar rats (initial weight 140–160 g) were given diets containing 0% (control), 1%, 4% and 8% of acacia gum (conformed to the British Pharmacopoeia specification) for 28 days (equivalent to 1,180, 4,720 and 9,440 mg acacia gum/kg bw per day, respectively). At autopsy, all organs of all animals were examined microscopically and some material was retained for electron microscopy and for microsomal cytochrome P450 assays. There were no detectable abnormalities in any of the organelles in the heart and the liver specimens. All histological observations were normal. The data from the assays of the liver microsomal protein and cytochrome P450 gave no indication for an inductive effect of acacia gum.

Cook et al. (1992) evaluated the oral toxicity of gum arabic (*Acacia* species not stated) using 3-week-old Sprague–Dawley rats (16 males and 16 females). Three days before dosing, mean body weights were 122 g and 125 g for males and females, respectively. The animals were fed gum arabic (dose not stated) daily for 28 days. Blood samples were obtained for haematological examination and serum analysis the day before animals were killed. After microscopic examination of organs, including any tissues that appeared abnormal, histology was normal. No treatment-related behavioural effects were noted. All values for serum chemistry parameters were within the normal limits for laboratory rats. Mean red blood cell volume values were within the normal historical control for rats in this laboratory.

Wistar albino rats (99–120 g) were fed a diet containing 10% gum arabic (*Acacia senegal* gum, conformed to the British Pharmacopoeia specification) (equivalent to 11,800 mg acacia gum/kg bw per day) daily for 45 days (Anderson et al., 1986). The number of rats in the study was not reported. Portions of the jejunum, ileum and caecum were excised, and examined using transmission electron microscopy. No abnormalities in organelles were observed within cells of the jejunum, ileum or caecum of rats fed gum arabic. Additionally, neither inclusions nor other pathological changes were detected. The authors concluded that there were no ultrastructural differences between treated and control rats.

Subchronic effects of acacia gum (80.8–85.5% purity) were also determined in F344 rats (initial weight 78–85 g) and B6C3F1 mice (initial weight 17–21 g) fed diets containing 0%, 0.63%, 1.25%, 2.5%, 5% or 10% acacia gum (reported as 100,000 mg acacia gum/kg diet) for 13 weeks (NTP, 1982). The dietary doses were equivalent to 560, 1,120, 2,250, 4,500 or 9,000 mg acacia gum/kg bw per day in rats and 1.25, 2.5, 5, 10 or 20 g acacia gum/kg bw per day in mice. Ten animals of each sex and each species per dose were used and separate control groups of each sex and species were included. The investigations included clinical signs, body weights, feed consumption and histopathology of all major organs. Haematology, clinical chemistry and urine were not investigated. No compound-related effects were observed in rats and mice, except a reduction in feed consumption at the two highest doses in males rats and at all doses in females rats as compared with the control animals (NTP, 1982).

Two 90-day-toxicity studies of acacia gum (conformed to the British Pharmacopoeia specification) performed in Wistar rats were reported (Anderson et al., 1982). In the first study, groups of 15 male and 15 female rats (24–28 days old) were fed for 13 weeks (90 days) at dietary concentrations of 0%, and around 1%, 2%, 4% and 8% equal to 0, 530, 1,080, 2,550 and 5,220 mg acacia gum/kg bw per day in males, and 0, 500, 1,050, 2,600 and 5,310 mg acacia gum/kg bw per day in females, respectively. The investigations included clinical signs, body weights, feed consumption, haematology, clinical chemistry, urinalysis, liver and kidney weights, and histopathology of all major organs. There was no reduction in the growth rate of male or female rats. There were no significant haematological

and urinalysis changes, and histopathology revealed no alterations. The authors concluded that there was no adverse effect up to the highest dose tested (5,000 mg acacia gum/kg bw per day).

In the second study, 15 male and 15 female rats per group were given for 13 weeks a diet containing acacia gum at 0% (control), 18.6% for males and 18.1% for females, in order to achieve a constant daily intake of approximately 14,000 mg acacia gum/kg bw per day. The same protocol as described above was performed. No haematological changes were reported, the only significant differences in serum were a decrease in serum total CO_2 and an increase in serum urea for female animals that received 14,000 mg acacia gum/kg bw per day; they had also a small reduction in kidney weight and caecal enlargement. In male rats receiving 14,000 mg acacia gum/kg bw per day, feed and water consumption, body weight, liver and kidney weights were significantly decreased and caecal enlargement was observed.

The Panel noted that an increased caecum weight in animals fed high amounts of carbohydrates is considered a physiological response to an increased fermentation. Increased caecum weight has been observed in rats fed carbohydrates other that acacia gum (Leegwater et al., 1974; Licht et al., 2006). Animals fed diets containing potato starch, inulin or oligofructose had significantly higher caecum weights and lower pH values than the reference animal group (Licht et al., 2006). Different groups of animals fed modified diets containing increased concentration of potato starch, hydroxypropyl starch and hydroxypropyl distarch glycerol showed increases in the relative caecal weights, filled and emptied, with increasing concentrations of the various hydroxypropyl starches. These increases were accompanied by increased severities of diarrhoea that was related to an increased osmotic activity of the caecal fluid in the animals (Leegwater et al., 1974). The authors hypothesised that dietary components not completely digested and/or absorbed in the small intestine, and further fermented by the gut microflora, enhance the amounts of osmotically active material resulting in an increase in water retention and the animals drinking more water leading to the caecum distention to a size larger than normal.

The Panel noted that these two studies were done independently and that merging their data may not be straightforward. The Panel noted that the only significant adverse effect reported in the second study, i.e. a decreased body weight gain in male rats, was due to the fact that the control group had a body weight gain well above the one of the control group reported in the first study. In this second study, male rats treated with the highest dose had a weight gain similar to the one of males from the control group in the first study. In addition, in the second study, the Panel considered that the high amounts of acacia gum given through the diet could have lead to a nutritional imbalance; therefore, the Panel considered that no relevant toxicological effects were observed in the two studies by Anderson et al. (1982). From the first study, the Panel identified a no observed adverse effect level (NOAEL) of 5,220 and 5,310 mg acacia gum/kg bw per day in male and female, respectively, the highest dose tested.

Doi et al. (2006) performed a 90-day study, under good laboratory practice (GLP), on a polysaccharide exudate from gum acacia trees (*A. senegal*) (purity, 100%). The compound was administered for 90 days in the diet to F344 rats (10 rats/sex per group) at levels of 0% (control), 1.25%, 2.5% and 5.0% (equal to 3,100 and 3,300 mg/kg bw per day in male and female rats, respectively). During the study, the treatment had no effects on clinical signs, survival, body weights, and feed and water consumption, or on findings of urinalysis, ophthalmology, haematology or blood biochemistry. Gross pathology and histopathology exhibited no differences of toxicological significance between control and treated rats. Increased relative caecum (filled) weights, evident in both sexes in the 5.0% groups and females in the 1.25% and 2.5% groups, were considered to be a physiological adaptation. The authors concluded that the NOAEL from the present study was 5.0% (3,100 mg/kg bw per day for males and 3,300 mg/kg bw per day for females). The Panel agreed with this NOAEL, the highest dose tested.

Overall, the short-term and subchronic administration of oral doses up to 5,000 mg acacia gum/kg bw per day to rats and 20,000 mg acacia gum/kg bw per day to mice did not induce any biologically relevant adverse effects. In some studies, caecal enlargement was observed. The Panel considered that an increased caecum weight in animals fed high amounts of carbohydrates is considered as a physiological response to an increased fermentation by the intestinal microbiota.

3.5.4. Genotoxicity

3.5.4.1. *In vitro*

In the study by Green (1977), acacia gum (unspecified origin) was assessed for its mutagenicity in the reverse mutation assay using *Salmonella* Typhimurium strains TA1530 and G-46 according to the method of Ames by the plate incorporation assay in the absence of rat liver S9 metabolism, and for mitotic recombination in *Saccharomyces cerevisiae* (strain D-3) in the absence of S9 metabolism only.

Negative results were reported for both mutagenic and mitotic recombination capabilities. However, the Panel noted that the study shows some shortcomings in the experimental design which include the use of a limited number of *S. typhimurium* strains, the absence of treatment in the presence of S9 metabolic activation and no indication of dose levels employed. On this basis, the Panel considered this study of limited value for risk assessment.

In the rec-assay employing the *Bacillus subtilis* strains M45 rec⁻, unable to repair DNA damage, and the wild-type strain H17 rec+ as control, acacia gum (unspecified origin) was assessed for its potential DNA-modifying effects at a single dose level of 10.3 mg/plate, both in the absence and presence of S9 metabolism. Negative results were obtained (Ishizaki and Ueno, 1987). The Panel noted that this mutagenicity assay is not frequently used and has not been validated.

In the study by Prival et al. (1991), acacia gum (origin Stein, Hall & Co.) was assessed for its mutagenicity in the reverse mutation assay using the *S. typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100, and the tryptophan-requiring *Escherichia coli* strain WP2, according to the method of Ames by the standard plate-incorporation assay both in the absence and presence of rat liver S9 metabolism up to dose levels of 10 mg/plate. Results obtained clearly indicated that acacia gum did not increase the number of revertant colonies both in the absence and presence of S9 metabolism. Furthermore, the Panel noted that the study complied with the current OECD Guideline 471.

Similarly, in the study by Zeiger et al. (1992), the mutagenicity of acacia gum (origin, Celanese Chemicals) was evaluated in the reverse mutation assay using the *S. typhimurium* strains TA 1535, TA1537, TA97, TA98 and TA100 according to the method of Ames by the pre-incubation protocol, both in the absence and presence of Aroclor 1254-induced rat and hamster S9 fractions at 10% and 30% up to dose level of 10 mg/plate. The outcome of the study clearly indicated that acacia gum was devoid of mutagenic activity under the reported experimental conditions. Furthermore, the Panel noted that the study complied with the current OECD Guideline 471 with the exception that tester strains TA102 or WP2uvrA bearing AT mutation were not used.

Newell and Maxwell (1972) and Maxwell and Newell (1974) assessed acacia gum (unspecified origin) for its ability to induce chromosomal aberrations in anaphase in the human embryonic lung cells (WI-38) at concentrations up to 1,000 μg/mL without S9 mix. Results reported by authors indicate slight increases in the frequency of cells bearing chromosomal aberrations particularly at the intermediate dose level used. However, the Panel noted that the observed increases were not dose-related and were accompanied by an elevated spontaneous level of chromosomal aberrations in the concurrent negative control (15.7%). In addition, the Panel noted that this assay did not receive further validation and is presently not used in genotoxicity testing.

Similarly, Green (1977) investigated the induction of chromosomal aberrations in anaphase by acacia gum (unspecified origin) in the human embryonic lung cells (WI-38) with questionable positive results, since no indication of dose levels employed and treatments performed only in the absence of S9 metabolic activation. However, the Panel noted that this assay did not receive further validation and is presently not used in genotoxicity testing.

3.5.4.2. *In vivo*

In the studies by Newell and Maxwell (1972) and Maxwell and Newell (1974), acacia gum (unspecified origin) was assessed for its genotoxicity in the following *in vivo* assays:

The host-mediated assay in Swiss Webster male mice administered once by oral gavage at 30, 2,500 and 5,000 mg/kg bw or for 5 consecutive days at the same dose levels employed in the single administration regime using the microbial systems *S. typhimurium* strains TA1530 and G-46 for mutagenicity and *S. cerevisiae* (strain D-3) for mitotic recombination.

Chromosomal aberrations in bone marrow cells of male albino rats administered by oral gavage once at 30, 2,500 and 5,000 mg/kg bw or for 5 consecutive days every 24 h at the same dose levels employed in the acute administration. In the acute treatment, sampling of bone marrow cells was performed at 6, 24 and 48 h after the last administration whereas, in the multiple administration study, sampling of bone marrow cells was only performed at 6 h from the last administration.

Dominant lethal assay in Sprague–Dawley rats following administration of the test compound by oral gavage once at 30, 2,500 and 5,000 mg/kg bw or for 5 consecutive days at the same dose levels employed in the single administration regime. Total implants (live fetuses plus early and late fetal deaths), total dead (early and late fetal deaths), dead implants per total implants and pre-implantation loss (calculated as the difference between the total corpora lutea and total implant counts) were evaluated.

The results reported indicated no effects for the host-mediated assay and dominant lethal assay, and 'slight positive findings' for the *in vivo* chromosomal aberration assay in bone marrow cells at the intermediate (5.3%) and high (5.2%) dose levels at the 6-h sampling time, compared with the concurrent negative control (0.7%). However, the Panel noted that increases in the number of aberrant cells observed at the 6-h sampling time at the intermediate and high dose levels were very similar to the incidence of aberrant cells observed in the negative control at the 24-h sampling time (4.0%) and were considered by the Panel to be of no biological significance. In addition, the Panel noted that the host-mediated assay did not receive further validation and is presently not used in genotoxicity testing.

In the study by Sheu et al. (1986), acacia gum (origin, Celanese Chemicals) was investigated for induction of chromosomal damage in rodent germ cells using the dominant lethal assay in male rats and dominant lethal and heritable translocation assays in mice. Acacia gum was incorporated into laboratory chow and fed to male rats and mice for 10 and 8 weeks, respectively. Three dose levels (500, 1,700 and 5,000 mg acacia gum/kg bw per day) were used. The treated male rats and mice were then tested for dominant lethal mutations evaluating the number of live and dead implants. The mice were also tested for induced heritable translocations. The authors reported negative results for both dominant lethal effects and heritable translocation in mice. Statistically significant increases for dominant lethal effects were instead observed in rats but considered to be of questionable biological significance by the authors. The Panel agreed with this conclusion and noted that increases of dead implants were small in absolute terms compared to the negative control values and were not dose-related.

Overall, the Panel noted that for the *in vivo* studies, acacia gum is not absorbed as such but appears, at the best, to be slightly fermented in the intestine to short chain fatty acids. On this basis the Panel considered the results of the *in vivo* studies of limited relevance for risk assessment.

In conclusion, the available *in vitro* and *in vivo* studies are generally limited or of limited relevance for different reasons. However, acacia gum was not mutagenic in *S. typhimurium* strains TA1535, TA1537, TA97, TA98, TA100 and TA 102 using the experimental method indicated by the OECD test guideline 471 (Prival et al. Zeiger et al., 1992) and did not show substantial evidence for the induction of chromosome mutations in mammalian cells *in vitro* in the anaphase chromosome aberration test, although this assay has not been validated and it is not currently employed for genotoxicity testing (Newell and Maxwell, 1972; Maxwell and Newell, 1974 and Green, 1977). Substantial negative results were also observed *in vivo* in the host-mediated assay in mice and the chromosomal aberration and dominant lethal assay in rats (Newell and Maxwell, 1972; Maxwell and Newell, 1974 and Sheu et al., 1986), although the relevance of these studies is limited due to the negligible absorption of the acacia gum.

Overall, based on the data available, the Panel concluded that there is no concern with respect to the genotoxicity of acacia gum.

3.5.5. Chronic toxicity and carcinogenicity

Mice and rats

No chronic toxicity studies were available.

A carcinogenicity study with acacia gum (80.8–85.5% purity) was conducted by feeding diets containing 25,000 mg/kg diet (2.5%) or 50,000 mg/kg diet (5%) of the test substance to 50 F344 rats and 50 B6C3F1 mice of each sex for 103 weeks. Groups of untreated rats and mice of each sex served as controls (NTP, 1982; Melnick et al., 1983). The dietary doses were equivalent to 1,250 and 2,500 mg acacia gum/kg bw per day in rats and 3,750 and 7,500 mg acacia gum/kg bw per day in mice. Throughout most of the study, mean body weights of dosed male and female mice and of dosed male rats were comparable with those of the controls; mean body weights of the dosed female rats were slightly lower than those of the controls. No other compound-related clinical signs or effects on survival were observed. Mean daily feed consumption of high-dosed rats and mice of either sex was 85–94% that of the controls.

According to NTP (1982), statistically significant ($p < 0.05$) increasing trends were observed for the number of female mice with hepatocellular carcinomas (1/49, 2/50, 6/50), and with total liver tumours (4/49, 2/50, 10/50). No statistically significant differences were obtained when comparing the control rates with those observed in the treated groups. These observations were not considered to be clearly associated with the dietary administration of acacia gum. Thus, no compound-related neoplastic or non-neoplastic lesions were found in rats or mice of either sex at doses up to 5% of acacia gum in the

diet, equivalent to 2.5 g acacia gum/kg bw per day in rats and 7.5 g acacia gum kg bw per day in mice. According to the authors, acacia gum was not carcinogenic in rat and mice. The Panel agreed with the conclusion of the authors and considered that acacia gum is not of concern with respect of carcinogenicity.

3.5.6. Reproductive and developmental toxicity

Reproductive and developmental toxicity of acacia gum was tested in different strains of mice and rats as well as in rabbits and hamsters.

3.5.6.1. Reproductive toxicity studies

In a combined fertility and developmental toxicity study of Collins et al. (1987) male and female Osborn–Mendel rats starting at 4 weeks of age. Body weights were recorded at regular intervals during the premating, mating and gestation period. Mating results were recorded by sperm detection. Pregnant (41–47) dams were observed daily for appearance and behaviour. The rats were treated from 13 weeks before mating, during gestation by specified acacia gum (A-12) added and blended with commercial diets. The rats were fed of 0%, 1%, 2%, 4%, 7.5 or 15% acacia gum in the diet (during gestation equal to 0, 683, 1,350, 2,836, 5,199 or 10,647 mg acacia gum/kg bw per day. At necropsy on gestation day (GD) 20, the numbers of implantation sites, resorption sites, live and dead fetuses, and body weights of live pups were recorded. All fetuses were examined grossly for external abnormalities, half for visceral examination and the other half only bone not cartilaginous stained and examined for skeletal defects). At doses up to 10,647 mg acacia gum/kg bw per day, there were no noticeable effects on pregnancy rate, implantation nor on maternal and fetal survival. The numbers of live or dead fetuses, resorptions, average implantations and fetal weights did not differ among the groups. The sex distribution of fetuses was not affected by the treatment. The number of abnormalities seen in either soft tissues or skeletons at fetal pathological examination of the acacia gum-treated groups did not differ from the number occurring spontaneously in vehicle-treated dams of the control group. There was no effect on female fertility. No adverse effects were mentioned about male fertility. The Panel identified from this study a NOAEL of for reproductive (fertility) effects of 10,647 mg acacia gum/kg bw per day, the highest dose tested.

Morseth and Ihara (1989a) evaluated the effect of a 5% solution of acacia gum (origin not specified) in water, on fertility and general reproductive performance using 30 male (6 weeks old, 181–226 g) and 30 female (10 weeks old, 210–309 g) Sprague–Dawley rats. The solution was administered by gavage once daily (5 mL/kg bw per day, equivalent to 250 mg acacia gum/kg bw per day) for 63 days prior to mating, throughout the mating period, and until the animals were killed. Male rats were killed after the females had littered. The oral dosing schedule for female rats was daily for 14 days prior to mating, throughout the mating period, and through GD 19 or 21 of lactation. Fifteen female rats were killed on day 20 of gestation, and the remaining females were allowed to raise their neonates to day 22 post-partum. No treatment–related abnormalities were observed in the oestrous cycles. Twenty-nine of the 30 females became pregnant; the male fertility index was 97%. Mean viability and mean weaning indices were 96% and 98%, respectively. No adverse effects were seen in this study at the only dose tested.

In a study to test the effects of another substance on fertility, 12 male Sprague-Dawley rats were fed 30% acacia gum (without further specifications; equivalent to 15,000 mg/kg bw per day) as vehicle controls (Huynh et al., 2000). Six males were killed after 82 days. During the last week, they could mate each two females. Following a period of up to 14 weeks, the remaining six males were killed and inspected. The following parameters were examined: fertility, including embryonic features in their mated females, hormone assays, blood and tissue examinations, epididymal sperm content and motility, sperm nuclear integrity and mitochondrial function. No effects were observed on mating behaviour and outcome, spermatogenesis, epididymal sperm function and fertility in male rats, at the end of the 82 days period and after the up to 14 weeks phase (Huynh et al., 2000). The Panel noted that in this fertility study acacia gum was used as control substance; the negative results cannot be used as a fertility assessment of acacia gum.

3.5.6.2. Developmental studies

In a study in mice receiving 5 oral doses of 0.5 mL of 1% and 10% (equivalent to 5 and 50 mg gum acacia gum/kg bw per day) solution of acacia gum (origin Merck AG) in water between day 11 and 15 of gestation, no embryotoxicity was observed (Frohberg et al., 1969).

Several developmental toxicity studies of acacia gum were conducted in Wistar rats, CD-1 mice, golden hamsters and Dutch belted rabbits (FDRL, 1972b). Animals were administered different doses of locust bean gum (not specified) suspended in anhydrous corn oil by gavage (1.0 mL/kg bw); the control groups were vehicle-treated. Body weights were recorded at regular intervals during gestation and all animals were observed daily for appearance and behaviour. All dams were subjected to caesarean section, and the numbers of implantation sites, resorption sites, live and dead fetuses, and body weight of live fetuses were recorded. All fetuses were examined grossly for external abnormalities, one-third underwent detailed visceral examinations and two-thirds were stained and examined for skeletal defects.

Mice

Pregnant CD-1 mice (19–21 animals/group) were treated by oral gavage once daily from GD 6 to 15 with doses of 0, 16, 75, 350 or 1,600 mg acacia gum/kg bw per day(no specification) in corn oil (20, 20, 21, 19 or 20 pregnant surviving females/group, respectively) (FDRL, 1972b). At necropsy on GD 17, the surviving dams appeared to be completely normal and the number of implantations, and live fetuses were comparable to the control group. Doses up to 1,600 mg acacia gum/kg bw per day had no noticeable effects on implantation nor on maternal and fetal survival. The numbers of live or dead fetuses, resorptions and the average implant sites, and also fetal weights did not differ among the groups. The sex distribution of fetuses was not affected by the treatment. The number of abnormalities seen in either soft tissues or skeletons at fetal pathological examination of the acacia gum-treated groups, did not differ from the number in vehicle-treated dams of the control group.

Rats

Pregnant Wistar rats (24 animals/group) were treated by oral gavage once daily from GD 6 to 15 with doses of 0, 16, 75, 350 or 1,600 mg acacia gum/kg bw per day in corn oil (23, 23, 22, 24 or 24 pregnant surviving females/group, respectively) (FDRL, 1972b). At necropsy on GD 20, doses up to 1,600 mg acacia gum/kg bw per day appeared to be completely normal and had no noticeable effects on implantation nor on maternal and fetal survival. The numbers of live or dead fetuses, resorptions, average implantations and fetal weights did not differ among the groups. The sex distribution of fetuses was not affected by the treatment. The number of abnormalities seen in either soft tissues or skeletons at fetal pathological examination of the acacia gum-treated groups did not differ from the number in vehicle-treated dams of the control group.

Hamsters

Pregnant Golden hamsters (20–22 animals/group) were treated by oral gavage once daily from GD 6 to 10 of gestation with doses of 0, 16, 75, 350 or 1,600 mg/kg bw per day of acacia gum in corn oil (20, 20, 19, 20 or 20 pregnant surviving females/group, respectively (FDRL, 1972b). At necropsy on GD 14, doses up to 1,600 mg acacia gum/kg bw per day appeared to be completely normal and showed no noticeable effects on implantation nor on maternal and fetal survival. The numbers of live or dead fetuses, resorptions, average implant sites or fetal weights did not differ among the groups. The sex distribution of fetuses was not affected by the treatment. The number of abnormalities seen in either soft tissues or skeletons at fetal pathological examination of the acacia gum-treated groups did not differ from the number in vehicle-treated dams of the control group.

Rabbits

Artificially inseminated Dutch-belted rabbits (15 animals/group) were treated by oral gavage once daily from GD 6 to 18 with doses of 0, 8, 37, 173 or 800 mg acacia gum/kg bw per day in corn oil (13, 11, 13, 9 or 8 pregnant surviving females/group, respectively) (FDRL, 1972b). The mortality in this test was 1, 2, 0, 3, 6 dams in the respective groups. Death was preceded by severe bloody diarrhoea, urinary incontinence and anorexia, 48–72 h before death, with, as pathological findings, haemorrhages in the mucosa of small intestines. At necropsy on GD 29, the surviving dams appeared normal throughout the observation period and had normal fetuses. No effect was observed on the number of implantations. The numbers of live or dead fetuses, resorptions, average implant sites or fetal weights did not differ among the groups. The sex distribution of fetuses was not affected by the treatment. The number of abnormalities seen in either soft tissues or skeletons at fetal pathological examination of the acacia gum-treated groups did not differ from the number in vehicle-treated dams of the control group. The higher maternal toxicity (lethality) observed in this study as compared to any of the other

species, can be caused by the difficulty of dosing rabbits by gavage with a viscous solution. Therefore, the Panel considered this study not suitable for the evaluation of risk assessment.

Male and female Osborne–Mendel rats were given diets containing 0% (control), 1%, 2%, 4%, 7.5% or 15% of acacia gum during premating, mating and throughout gestation (Collins et al., 1987). During gestation, the treated females consumed from 683 mg acacia gum/kg bw per day in the 1% group to 10,647 mg acacia gum/kg bw per day in the 15% group. The animals were killed on GD 20. There were no dose-related changes in maternal findings, number of fetuses, fetal viability or external, visceral or skeletal variations. The Panel identified from this study a NOAEL of for developmental effects of 10,647 mg acacia gum/kg bw per day, the highest dose tested.

Morseth and Ihara (1989b) investigated the developmental effects of a 5% solution of acacia gum in water using 37 female Crl:CDBR rats (9 months old, 207–314 g) for which mating had been confirmed. The solution was administered by gavage once daily (5 mL/kg per day, equivalent to 250 mg acacia gum/kg bw per day) from GD 6 to 17. Dams selected to study the developmental effects (24 females with caesarean section) were necropsied on day 20 of gestation. Fetuses were subjected to external (weight, examination of external abnormalities), visceral and skeletal (skull, long bones, vertebral column, rib cage, extremities, girdles) examinations. In females subjected to natural delivery (13 animals), one pup/sex per litter was subjected to behavioural evaluation, whereas the first male and female of each litter were used for breeding and measuring of reproductive ability. External, visceral or skeletal variations were not observed in any of the fetuses evaluated. There were no effects in the post-weaning behavioural evaluation or growth ratios. There were no treatment-related effects in F_1 reproductive indices and growth of F_2 pups.

Overall, in a dietary combined fertility and developmental toxicity study in rats (Collins et al., 1987) a NOAEL of 10,647 mg acacia gum/kg bw per day for reproductive, developmental and parental effects was identified, the highest dose tested. In addition, other reproductive studies in rats showed no effects at the highest dose tested (Morseth and Ihara (1989a), Huynh et al., 2000). In the identically performed prenatal developmental tests with acacia gum by gavage in mice, rats and hamsters (FDRL, 1972b), 1,600 mg/kg bw per day (the highest doses tested) showed no dose-related developmental effects.

3.5.7. Hypersensitivity, allergenicity and food intolerance

The immunogenicity of acacia gum was compared to that of other gums in inbred mice (Strobel et al., 1982). The gums were dissolved in 0.15 M NaCl at a concentration of 4 mg/mL. Mice (6–8 per group, aged 6 weeks) were immunised with antigen emulsified in complete Freund's adjuvant. Twenty-one days after primary immunisation, the presence of delayed-type hypersensitivity was measured by a skin test and the specific cell-mediated immunity subsequently measured by a footpad swelling test. The immune response of acacia gum was comparable to that elicited by other common foodstuff components, e.g. hen's ovalbumin.

Serum immunoglobulin E (IgE) antibodies specific for the carbohydrate moiety of acacia gum and cross reactive with the carbohydrates found in pollens have been detected in a patient with strong respiratory allergy to acacia gum (Fötisch et al., 1998). In another study on one person occupationally exposed to acacia gum dust, the authors suggested that allergy to acacia gum was mediated preferentially by IgE antibodies directed to the polypeptide chains of acacia gum (Sander et al., 2006). Occupational sensitisation to acacia gum has been described after atmospheric exposure at work (Viinanen et al., 2011).

No case reports on allergic reaction after oral exposure to acacia gum could be identified by the Panel.

3.5.8. Other studies

3.5.8.1. Human data

Five healthy male volunteers (33–55 years old) ingested daily doses of 25 g acacia gum (approx. 350 mg acacia gum/kg bw per day, as a solution in 7% dextrose, conformed to the British Pharmacopoeia specification) for 21 days (Ross et al., 1983). The dose was well tolerated. Several haematological and biochemical parameters, glucose absorption and biological assays of components of urine and faeces were measured. Acacia gum had no effect on haematology and serum biochemistry after a 3-week daily administration. Only a minimum effect on glucose tolerance, stool weight and decreased serum cholesterol were observed. The Panel noted that there were no side effects in this study.

Sharma (1985) described a reduction of low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) cholesterol by approximately 10% when acacia gum was given to 2 × 15 g/person per day and to seven individuals for 30 days without having effects on high-density lipoprotein (HDL) cholesterol and triglycerides. In this report, from the seven individuals treated, a few experienced flatulence after ingestion of the gum.

In a study with 10 volunteers (4 men and 6 women, aged 22/33 years) treated for 10 days, dosages of 15,000 mg acacia gum/person per day increased water excretion with the stool and 10,000 mg acacia gum/person per day and 15,000 mg acacia gum/person per day increased the counts and proportion of bifidobacteria in human stools (Cherbut et al., 2003). In a second study by the same authors, 10 subjects (5 men and 5 women, aged 22/38 years) received 10–70 g of acacia gum per day (divided in two to six doses per day) in an escalation schedule lasting 18 days. The authors report that dosages below 30,000 mg acacia gum/person per day (approximately equivalent 430 mg acacia gum/kg bw per day) did not induce flatulence, while a dose of 53,000 mg acacia gum/person per day (approximately equivalent 760 mg acacia gum/kg bw per day) induced mild flatulence but did not provoke abdominal cramps or diarrhoea (Cherbut et al., 2003).

4. Discussion

Acacia gum is a dried exudation obtained from the stems and branches of natural strains of *A. senegal* (L.) Willdenow or closely related species of *Acacia* (family Leguminosae) (JECFA, 2006).

Specifications for acacia gum (E 414) have been defined in Commission Regulation (EU) 231/2012. The Panel noted that according to the EC specifications it is not clear which are the closely related species of acacia, while in the JECFA specifications (Documentation provided to EFSA, n.5), it is indicated that gum arabic (acacia gum) can be obtained from *A. senegal* (L.) Willdenow or *A. seyal* (family Leguminosae). The Panel noted that the EC specifications do not limit the protein content which according to Phillips et al. (2008) can be between 0.13% and 10.4%. According to industry (AIPG 2015), contents of proteins were in a range from 0.99% to 2.70% as determined in three samples analysed in duplicate. The Panel agreed with the proposal by interested parties to include a limit for protein content of 3.5% in the EC specifications.

Because of both the botanical origin and the polysaccharidic nature of gums, they can be a substrate of microbiological contamination and of field and storage fungal development. The latter has been recently demonstrated by the mycotoxin contaminations of gums (Zhang et al., 2014). The Panel noted that the microbiological specifications for polysaccharidic thickening agents, such as gums, should be harmonised and that for acacia gum criteria for TAMC and TYMC should be included into the EU specifications.

Regarding the possible presence of nanoparticles in the dry powder of acacia gum resulting from the manufacturing process, the Panel considered that the material used for toxicological testing would contain this nanofraction, if present. In addition, the Panel noted that contact of acacia gum with any liquids (in food or biological fluids) will result in an increase of the particle size.

The *in vitro* degradation and the *in vivo* digestibility of acacia gum have been investigated in animals and humans models and in a human study. The Panel considered that these data indicated that acacia gum would be not absorbed intact but fermented by enteric bacteria in humans. The rate of hydrolysis in the gastrointestinal tract in humans is unknown; however, the Panel considered that acacia gum is unlikely to be absorbed intact, and that the limited extent of its fermentation would lead to products such as SCFA which were considered of no safety concern by the Panel.

Acacia gum is regarded as having a low acute oral toxicity.

In a subacute toxicity study (Anderson et al., 1984), no histopathological changes were identified by electron microscopic examination of organs from rats fed diets containing 1–8% acacia gum daily (equivalent to 1,180–9,440 mg acacia gum/kg bw per day) for 28 days.

Among other studies, the subchronic (13 weeks) oral toxicity of acacia gum was investigated by Anderson et al. (1982). The animals received acacia gum in their diet and the study was conducted in two consecutive experimental phases. In the first one, the rats were given doses ranging from 0 to about 5,000 mg acacia gum/kg bw per day and in the second phase, they received 0 or 14,000 mg acacia gum/kg bw per day. The Panel noted that these two studies were done independently and that merging their data may not be straightforward. The Panel considered that no toxicological effect was observed in these studies by Anderson et al. (1982). From the first study, no adverse effects have been identified up to 5,220 and 5,310 mg acacia gum/kg bw per day in male and female, respectively, the highest dose tested.

Overall, the short-term and subchronic administration of oral doses up to 5,000 mg acacia gum/kg bw per day to rats and 20,000 mg acacia gum/kg bw per day to mice, the highest doses tested, did not induce any biologically relevant adverse effects. In some studies, caecal enlargement was observed. The Panel considered that an increased caecum weight in animals fed high amounts of carbohydrates is considered as a physiological response to an increased fermentation by the intestinal microbiota.

Based on the data available, the Panel considered that there is no concern with respect to the genotoxicity of acacia gum.

No chronic toxicity studies according to OECD guidelines (452) or equivalent have been identified.

Acacia gum was tested for carcinogenicity in rats and mice receiving diets containing 2.5% and 5% acacia gum in the feed for 103 weeks equivalent to 1,250 and 2,500 mg acacia gum/kg bw per day in rats, and 3,750 and 7,500 mg acacia gum/kg bw per day in mice (NTP, 1982; Melnick et al., 1983). From this study, the Panel considered that acacia gum is not of concern with respect to carcinogenicity.

In a dietary combined fertility and developmental toxicity study in rats (Collins et al., 1987), a NOAEL of 10,647 mg acacia gum/kg bw per day for reproductive, developmental and parental effects was identified, the highest dose tested. In addition, other reproductive studies in rats showed no effects at the highest dose tested (Morseth and Ihara (1989a), Huynh et al., 2000). In the identically performed prenatal developmental tests with acacia gum by gavage in mice, rats and hamsters (FDRL, 1972b), 1,600 mg/kg bw per day (the highest doses tested) showed no dose-related developmental effects.

No case reports on allergic reaction after oral exposure to acacia gum could be identified by the Panel.

In humans, the repeated oral daily intake of a large amount of acacia gum up to 30 g (approx. 430 mg acacia gum/kg bw per day) for up to 18 days was well tolerated and had only a minimum effect on stool weight and decrease in serum cholesterol. Some individuals experienced flatulence which was considered by the Panel as undesirable but not adverse.

To assess the dietary exposure to acacia gum (E 414) from its use as a food additive, the exposure was calculated based on (1) maximum levels of data provided to EFSA (defined as the *maximum level exposure assessment scenario*) and (2) reported use levels (defined as the *refined exposure assessment scenario, brand-loyal* and *non-brand-loyal consumer scenario*).

Acacia (E 414) is authorised in a wide range of foods. The Panel did not identify brand loyalty to a specific food category, and therefore, the Panel considered that the non-brand-loyal scenario covering the general population was the more appropriate and realistic scenario for risk characterisation because it is assumed that the population would probably be exposed long-term to the food additive present at the mean reported use in processed food.

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

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

The refined estimates are based on 31 out of 76 food categories in which acacia gum (E 414) is authorised. The Panel considered that the uncertainties identified would, in general, result in an overestimation of the exposure to acacia gum (E 414) as a food additive in European countries for the refined scenario if it is considered that the food additive may not be used in food categories for which no usage data have been provided. However, the Panel noted that given the information from the Mintel's GNPD, it may be assumed that acacia gum (E 414) is used in food categories for which no data have been provided by food industry.

The main food categories, in term of amount consumed, not taken into account were unflavoured fermented milk products, cheeses, breakfast cereals, foods for infants and young children (processed cereal-based foods and baby food, other foods for young children), snacks and some alcoholic beverages (cider and perry, spirit drinks, etc.). According to the Mintel GNPD (Appendix C), in the EU market, snacks and breakfast cereals are labelled with acacia gum (E 414), as well as few alcoholic

drinks and nectars. Therefore, the Panel considered that if these uncertainties were confirmed, it would therefore result in an underestimation of the exposure.

The Panel noted that in Annex II of Regulation (EC) No 1333/2008, use levels of acacia gum (E 414) in food for infants under the age of 12 weeks are included in category 13.1.5.2. The Panel considered that these uses would require a specific risk assessment in line with the recommendations given by JECFA (1978) and the SCF (1998) and endorsed by the Panel (EFSA ANS Panel, 2012). Therefore, the current re-evaluation of acacia gum (E 414) as a food additive is not considered to be applicable for infants under the age of 12 weeks and will be performed separately.

The Panel further noted that the exposure to acacia gum from its use according the Annex III (Part 1, 2, 3, 4 and 5) was not considered in the exposure assessment.

The Panel also noted that the refined exposure estimates are based on information provided on the reported level of use of acacia gum (E 414). If actual practice changes, this refined estimates may no longer be representative and should be updated.

5. Conclusions

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

- the safety assessment carried out by the Panel is limited to the use and use levels in 31 out of 76 food categories in which acacia gum (E 414) is authorised (refined exposure assessment scenario);
- an indicative high refined exposure assessment up to 719 mg/kg bw per day has been calculated in toddlers at the 95th percentile (non-brand loyal scenario) for the general population;
- an indicative high refined exposure assessment up to 626 mg/kg bw per day has been calculated in toddlers at the 95th percentile in the brand loyal scenario for the population consuming FSMPs;
- acacia gum is unlikely to be absorbed intact and is slightly fermented by intestinal microbiota;
- sufficient toxicity data were available;
- there is no concern with respect to the genotoxicity;
- no carcinogenic effects were reported in carcinogenicity studies in mice and rats at the doses up to 7,500 mg and 2,500 mg acacia gum/kg bw per day, respectively, the highest doses tested;
- oral daily intake of a large amount of acacia gum up to 30,000 mg acacia gum/person per day (approximately equivalent 430 mg acacia gum/kg bw per day) for up to 18 days was well tolerated in adults but some individuals experienced flatulence. A dose of 53,000 mg acacia gum/person per day (equivalent to 760 mg acacia gum/kg bw per day) induced mild flatulence, which was considered by the Panel as undesirable but not adverse,

the Panel concluded that there is no need for a numerical ADI for acacia gum (E 414), and that there is no safety concern at the refined exposure assessment for the reported uses of acacia gum (E 414) as food additive.

6. Recommendations

The Panel noted that currently detected levels of these toxic elements (lead, cadmium, mercury and arsenic) were far below those defined in the EC specifications for acacia gum, and therefore, the current limits should be lowered in order to ensure that acacia gum (E 414) as a food additive will not be a significant source of exposure to those toxic elements in food, in particular for infants and children. The Panel also recommended that limits for aluminium should be included in the EC specifications.

The Panel recommended to harmonise the microbiological specifications for polysaccharidic thickening agents, such as gums, and to include criteria for TAMC and TYMC into the EU specifications of acacia gum.

The Panel recommended that the oxidases and peroxidases in acacia gum should be inactivated during the manufacturing process to avoid any oxidative degradation of components in preparations to which acacia gum is added. The Panel further recommended limits for residual enzymatic activities and for protein content in the EC specifications.

Due to the discrepancies observed between the data reported from industry and the Mintel database, where acacia gum (E 414) is labelled in more products than in food categories for which data were reported from industry, the Panel recommended collection of data of usage and use levels of acacia gum (E 414) in order to perform a more realistic exposure assessment.

Documentation provided to EFSA

1) MARS Chocolate UK. Data submitted to EFSA on 19 May 2010.
2) Pre-evaluation document prepared by Fraunhofer-Gesellschaft zur Förderung der angewandten Forschung e.V. October 2011.
3) Association for International Promotion of Gums (AIPG). Data submitted to EFSA on 4 March 2010.
4) Association for International Promotion of Gums (AIPG). Data submitted to EFSA on 24 April 2013.
5) Association for International Promotion of Gums (AIPG). Data submitted to EFSA on 22 December 2015.
6) Association for International Promotion of Gums (AIPG). Data submitted to EFSA on 16 February 2016.
7) Riemser Arzneimittel AG. E 414 Gummi arabicum. Data submitted 25 April 2013.
8) EMA (European Medicines Agency): communication to EFSA request in 4 May 2015, for information on a certain group of substances used as food additives, June 2014.
9) AIPG (Association for international Promotion of Gums), 2014. Data on usage levels of acacia gum (E 414) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2014). Submitted to EFSA on 17 September 2014.
10) FDE (FoodDrinkEurope), 2013. Data on usage levels of acacia gum (E 414) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2014). Submitted to EFSA on 29 November 2014.
11) Interested party 1, 2014. Data on usage levels of acacia gum (E 414) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2014). Submitted to EFSA on 4 July 2014.
12) Stollwerck GMBH., 2014. Data on usage levels of acacia gum (E 414) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2014). Submitted to EFSA on 28 August 2014.
13) DOMACO (DOMACO Dr. med. Aufdermaur AG), 2014. Data on usage levels of acacia gum (E 414) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2014). Submitted to EFSA on 30 July 2014.
14) F. Hunziker & CO, 2014. Data on usage levels of acacia gum (E 414) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2014). Submitted to EFSA on 4 September 2014.
15) A.H. Meyer & Cie AG, 2014. Data on usage levels of acacia gum (E 414) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2014). Submitted to EFSA on 31 July 2014.
16) ICGA (International Chewing Gum Association), 2014. Data on usage levels of acacia gum (E 414) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2014). Submitted to EFSA on 30 September 2014.
17) ASSICA (Associazione Industriali delle Carni e dei Salumi), 2014. Data on usage levels of acacia gum (E 414) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2014). Submitted to EFSA on 25 September 2014.
18) SNE (Specialised Nutrition Europe), 2014. Data on usage levels of acacia gum (E 414) in foods in response to the EFSA call for food additives usage level and/or concentration data

in food and beverages intended for human consumption (2014). Submitted to EFSA on 30 September 2014.

19) CHEPLAPHARM Arzneimittel GmbH, 2014. Data on usage levels of acacia gum (E 414) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2014). Submitted to EFSA on 21 August 2014.
20) Rudolf Wild GmbH & Co. KG, 2014. Data on usage levels of acacia gum (E 414) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2014). Submitted to EFSA on 29 September 2014.
21) Frutarom Industries Ltd, 2014. Data on usage levels of acacia gum (E 414) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2014). Submitted to EFSA on 26 September 2014.
22) Nathura, 2014. Data on usage levels of acacia gum (E 414) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2014). Submitted to EFSA on 19 September 2014.
23) AESGP (Association of the European Self-Medication Industry), 2013. Data on usage levels of acacia gum (E 414) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2014). Submitted to EFSA on 9 September 2013.

References

Adiotomre J, Eastwood MA, Edwards CA and Brydon WG, 1990. Dietary fibre: *in vitro* methods that anticipate nutrition and metabolic activity in humans. American Journal of Clinical Nutrition, 52, 128–134.

Anderson DMW, Ashby P, Busuttil A, Eastwood MA, Hobson BM, Ross AHM and Street CA, 1982. Subchronic effects of gum arabic (Acacia) in the rat. Toxicology Letters, 14, 221–227.

Anderson DMW, Ashby P, Busuttil A, Kempson SA and Lawson ME, 1984. Transmission Electron Microscopy of heart and liver tissues from rats fed with gum arabic. Toxicology Letters, 21, 83–89.

Anderson DMW, Howlett JF and McNab CGA, 1985. The amino acid composition of the proteinaceous component of gum arabic (*Acacia senegal* (L.) Willd). Food Additives and Contaminants, 2, 159–164.

Anderson DMW, Busuttil A, Kempson SA and Penman DW, 1986. Transmission electron microscopy of jejunum, ileum, and caecum tissues from rats fed with gums Arabic, karaya and tragacanth. Toxicology, 41, 75–82.

AOAC (Association of Analytical Communities), 1998. AOAC Official method 993.19: soluble Dietary Fiber in Food and Food Products. In: Horwitz W (ed.). *Official methods of Analysis of AOAC International*, Vol. II. 17th edition. pp. 80–81.

Billaud C, Lecornu D and Nicolas J, 1996. Substrates and carboxylic acid inhibitors of partially purified polyphenol oxidase from gum arabic. Journal of Agriculture and Food Chemistry, 44, 1668–1675.

Booth AN, Hendrickson AP and Deeds F, 1963. Physiologic effects of three microbial polysaccharides on rats. Toxicology and Applied Pharmacology, 5, 478–484.

Bracher F, Heisig P, Langguth P, Mutschler E, Rücker G, Scriba G, Stahl-Biskup E and Troschütz R, 2005. *Kommentar zum Europäischen Arzneibuch (Commentary to the Pharmacopoeia Europaea)*. Wissenschaftliche Verlagsgesellschaft, Stuttgart, Germany.

Cherbut C, Michel C, Raison V, Kravtchenko T and Severine M, 2003. Acacia gum is a bifidogenic dietray fibre with high digestive tolerance in healthy humans. Microbial Ecology in Health and Disease, 15, 43–50.

CIR, 2005. Final Report of 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, 24(Suppl. 3), 75–118.

Collins TFX, Welsh JJ, Black TN, Graham SL and Brown LH, 1987. Study of teratogenic potential of gum arabic. Food and Chemical Toxicology, 25, 815–821.

Cook WM, Purchase R, Ford GP, Creasy DM, Brantom PG and Gangolli D, 1992. A 28-day feeding study with ethyl acetoacetate in rats. Food and Chemical Toxicology, 30, 567–573.

Crociani F, Alessandrini A, Mucci MM and Biavati B, 1994. Degradation of complex carbohydrates by *Bifidobacterium* spp. International Journal of Food Microbiology, 24, 199–210.

Cummings JH and Englyst HN, 1987. Fermentation in the human large intestine and the available substrates. American Journal of Clinical Nutrition, 45, 1243–1255.

Den Besten G, van Eunen K, Groen A, Venema K, Reijngoud DJ and Bakker M, 2013. The role of short-chain fattyacids in the interplay between diet, gut microbiota, and host energy metabolism. The Journal of Lipid Research, 54, 2325–2340.

Doi Y, Ichihara T, Hagiwara A, Imai N, Tamano S, Orikoshi H, Ogasawara K, Sasaki Y, Nakamura M and Shirai T, 2006. A ninety-day oral toxicity study of a new type of processed gum arabic, from Acacia tree (*Acacia senegal*) exudates, in F344 rats. Food and Chemical Toxicology, 44, 560–566.

Dror Y, Cohen Y and Yerushalma-Rozen, R, 2006. Structure of Gum Arabic in aqueous solution. Journal of Polymer Science: Part B: Polymer Physics, 44, 3265–3271.

EFSA (European Food Safety Authority), 2007. Opinion of the Scientific Committee related to uncertainties in dietary exposure assessment. EFSA Journal 2007;5(1):438, 54 pp. doi:10.2903/j.efsa.2007.438

EFSA (European Food Safety Authority), 2011a. Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. EFSA Journal, 2011;9(3):2097, 34 pp. doi:10.2903/j.efsa.2011.2097

EFSA (European Food Safety Authority), 2011b. Evaluation of the FoodEx, the food classification system applied to the development of the EFSA Comprehensive European Food Consumption Database. EFSA Journal 2011;9 (3):1970, 27 pp. doi:10.2903/j.efsa.2011.1970

EFSA AFC Panel (EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food), 2004. Opinion on a request from the Commission related to the use of certain food additives in jelly mini cups. EFSA Journal 2004;8(2):82, 11 pp. doi:10.2903/j.efsa.2004.82

EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), 2008. Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Food Contact Materials on a request from European Commission on Safety of aluminium from dietary intake. EFSA Journal 2008;6(7):754, 1–34. doi: 10.2903/j.efsa.2008.754

EFSA ANS Panel (Panel on Food Additives and Nutrient Sources added to Food), 2010. Scientific Opinion on the use of Gum Acacia modified with Octenyl Succinic Anhydride (OSA) as a food additive. EFSA Journal 2010;8 (3):1539, 23 pp. doi:10.2903/j.efsa.2010.1539

EFSA ANS Panel (European Food Safety Authority Panel on Food Additives and Nutrient Sources added to Food), 2012. Guidance for submission for food additive evaluations. EFSA Journal 2012;10(7):2760, 60 pp. doi: 10.2903/j.efsa.2012.2760. Available online: http://www.efsa.europa.eu/efsajournal

EFSA ANS Panel (EFSA ANS Panel on Food Additives and Nutrient Sources added to Food), 2014. Statement on a conceptual framework for the risk assessment of certain food additives re-evaluated under Commission Regulation (EU) No 257/2010. EFSA Journal 2014;12(6):3697, 11 pp. doi:10.2903/j.efsa.2014.3697

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2009a. Scientific Opinion on cadmium in food. EFSA Journal 2009;7(10):980, 139 pp. doi:10.2903/j.efsa.2009.980

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2009b. Scientific Opinion on arsenic in food. EFSA Journal 2009;7(10):1351, 199 pp. doi:10.2903/j.efsa.2009.1351

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2010. Scientific Opinion on lead in food. EFSA Journal 2010;8(4):1570, 151 pp. doi:10.2903/j.efsa.2010.1570

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2012. Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. EFSA Journal 2012;10(12):2985, 241 pp. doi:10.2903/j.efsa.2012.2985

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2010. Scientific Opinion on the substantiation of health claims related to acacia gum (gum arabic) and reduction of post-prandial glycaemic responses (ID 842, 1977) and maintenance of normal blood glucose concentrations (ID 842, 1977) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Journal 2010;8(2):1475, 14 pp. doi:10.2903/j.efsa.2010. 1475. Available online: www.efsa.europa.eu

EFSA Scientific Committee, 2009. Guidance of the Scientific Committee on Transparency in the Scientific Aspects of Risk Assessments carried out by EFSA. Part 2: general Principles. EFSA Journal 2009;7(7):1051, 22 pp. doi: 10.2903/j.efsa.2009.1051

EFSA Scientific Committee, 2012. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 2012;10(3):2579, 32 pp. doi:10.2903/j.efsa.2012.2579

European Pharmacopoeia, 2015. *Acaciae gummi*, 8.5th Edition. pp. 7168–7169.

FAO, (Food and Agriculture Organization of the United Nations), 1999. Compendium of food additive specifications. Addendum 7 (Joint FAO/World Health Organization Expert Committee on Food Additives. 53rd Session), 1–2 Rome: FAO. Available online: http://www.fao.org/docrep/x3860e/X3860E20.htm#P5107_110391

FDRL (Food and Drug Research Laboratories), 1972a. Acute toxicity studies on gum arabic. Unpublished. Food and Drug Research Laboratories, Inc. Contract No. FDA 71-260.

FDRL (Food and Drug Research Laboratories), 1972b. Teratologic evaluation of FDA 71-15 (gum Arabic) gum in mice, rats, hamsters and rabbits. Rep.: FDABF-GRAS-063 Aug 1972.

Fenyo JC and Vandevelde MC, 1990. Physico-chemical properties of gum arabic in relation to structure. In *"Gum and Stabilizer for the Food Industry"*. Vol. 5. IRL Press, Oxford. pp. 17–23.

Fötisch K, Fäh J, Wüthrich B, Altman F, Haustein D and Vieths S, 1998. IGE antibodies specific for carbohydrates in a patient allergic to gum arabic (*Acacia senegal*). Allergy, 53, 1043–1051.

Frohberg H, Oettel H and Zeller H, 1969. Mechanism of the fetotoxic effect of tragacanth. Archiv für Toxikologie (Archives of Toxicology), 25, 268–295.

Glicksman M and Sand RE, 1973. Gum Arabic. In: Whistler RL (ed.). *"Industrial Gums. Polysaccharides and Their Derivatives"*. Academic Press: NY. pp. 197–265.
Goodrum LJ, Patel A, Leykam JF and Kieliszewski MJ, 2000. Gum arabic glycoprotein contains glycomodules of both extension and arabinogalactan-glycoproteins. Phytochemistry, 54, 99–106.
Green S, 1977. Present and future uses of mutagenicity tests for assessment of the safety of food additives. Journal of Environmental Pathology, Toxicology and Oncology, 1, 49–54.
Huynh PN, Hikim APS, Wang C, Stefonovic K, Lue YH, Leung A, Atienza V, Baravarian S, Reutrakul V and Swerdloff RS, 2000. Long-term effects of triptolide on spermatogenesis, epididymal sperm function, and fertility in male rats. Journal of Andrology, 21, 689–699.
Idris OHM, Williams PA and Phillips GO, 1998. Characterisation of gum from *Acacia senegal* trees of different age and location using multidetection gel permeation chromatography. Food Hydrocolloids, 12, 379–388.
Informatics Inc., 1972. GRAS (Generally Recognized as Safe) food ingredients – gum arabic. NTIS Report No. PB 223 614.
IPCS, 2004. IPCS Risk Assessment Terminology (Part 1: IPCS/OECD Key Generic terms used in Chemical Hazard/ Risk Assessment. Available online: http://www.inchem.org/documents/harmproj/harmproj/harmproj1.pdf
Ishizaki M and Ueno S, 1987. The DNA-damaging activity of natural food additives (IV) Shokuhin Eiseigaku Zasshi. Journal of the Food Hygiene Society of Japan, 28, 498–501.
Islam AM, Phillips GO, Sljivo A, Snowden MJ and Williams PA, 1997. A review of recent developments on the regulatory, structural and functional aspects of gum arabic. Food Hydrocolloids, 11, 493–505.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1972. WHO Technical Report Series, evaluation of certain food additives and the contaminants mercury, lead, and cadmium. Sixteenth report of the Joint FAO/ WHO Expert Committee on Food Additives. No. 505. World Health Organization, Geneva.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1978. Evaluation of certain food additives. Twenty-first report of the Joint FAO/WHO Expert Committee on Food Additives. No. 617. World Health Organization, Geneva.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1982. Toxicological evaluation of certain food additives, Series number 17, ID number 526. Arabic gum (gum arabic). Twenty-sixth Report of the Joint FAO/ WHO Expert Committee on Food Additives, WHO Food Additive Series 17. Available online: http://www.inchem.org/documents/jecfa/jecmono/v17je06.htm
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1990. Toxicological evaluation of certain food additives and contaminants, Series number 26, ID number 686. Gum arabic. Thirty-fifth Report of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additive Series 26. Available online: http://www.inchem.org/documents/jecfa/jecmono/v26je07.htm
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1998. Joint FAO/WHO Expert Committee on Food Additives. Fifty-first meeting Geneva, 9-18 June 1998. Available online: http://www.leffingwell.com/Summary%20and%20Conclusions%20of%20the%20Fifty-first%20Meeting.pdf
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2000. Guidelines for the preparation of toxicological working papers for the Joint FAO/WHO Expert Committee on Food Additives. Geneva, Switzerland.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2006. Gum Arabic. Joint FAO/WHO Expert Committee on Food Additives. Available online: http://www.fao.org/ag/agn/jecfa-additives/details.html?id=766
Kishimoto A, Ushida K, Phillips GO, Ogasawara T and Sasaki Y, 2006. Identification of intestinal bacteria responsible for fermentation of gum arabic in pig model. Current Microbiology, 53, 173–177.
Klose RE and Glicksman M, 1990. *CRC Handbook of Food Additives*. Chapter 7, Vol. I, 2nd Edition. In: Furia TE (ed.). CRC Press, Inc., NW, Bocca Raton, Florida, US.
Leegwater DC, de Groot AP and van Kalmthout-Kuyper M, 1974. The aetiology of caecal enlargement in the rat. Food and Cosmetics Toxicology, 12, 687–697.
Leung AY and Foster S, 2003. *Acacia in Encyclopedia of natural ingredients used in food, drugs and cosmetics*, 2nd Edition. John Wiley and sons, Hoboken, New Jersey.
Licht TR, Hansen M, Poulsen M and Dragsted LO, 2006. Dietary carbohydrate source influences molecular fingerprints of the rat faecal microbiota. BMC Microbiology, 6, 98–107.
Mahendran T, Williams PA, Phillips GO and Al-Assaf Sand Baldwin TC, 2008. New insights into the structural characteristics of the arabinogalactan-protein (AGP) fraction of gum Arabic. Journal of Agricultural and Food Chemistry, 56, 9269–9276.
Mallett AK, Wise A and Rowland IR, 1984. Hydrocolloid food additives and rat caecal microbial enzyme activities. Food and Chemical Toxicology, 22, 415–418.
Martindale, 2014. *The Complete Drug Reference*, 38th Edition, Pharmaceutical Press, London, Acacia gum.
Martindale, 2016. The complete drug reference. Acacia; online. Pharmaceutical Press, London. Date of monograph revision: 8-Mar-2016. Available online: http://www.medicinescomplete.com/mc/martindale/current/
Maxwell WA and Newell GW, 1974. Screening techniques for environmental mutagens. In: *Molecular & Environmental Aspects of Mutagenesis*. 6th Rochester International Conference on Environmental Toxicity. Prakash Louise Ed., 14, 223–252.

Melnick RL, Huff J, Haseman JK, Dieter MP, Grieshaber CK, Wyand DS, Russfield AB, Murthy ASK, Fleischman RW and Lilja HS, 1983. Chronic effects of agar, guar gum, gum arabic, locust bean gum, or tara gum in F344 rats and B6C3F1 mice. Food and Chemical Toxicology, 21, 305–311.

Monke JV, 1941. Non-availability of gum acacia as a glycogenic foodstuff in the rat. Proceedings of the Society for Experimental Biology and Medicine, 46, 178–179.

Morseth SL and Ihara T, 1989a. Reproduction study in rats with manidipine hydrochloride (CV-4093 (2 HCl)). Yakuri To Chiryo (Japanese Pharmacology and Therapeutics), 17, 145–162.

Morseth SL and Ihara T, 1989b. Teratology study in rats with manidipine hydrochloride (CV-4093 (2 HCl)). Yakuri To Chiryo (Japanese Pharmacology and Therapeutics), 17, 163–183.

Newell GW and Maxwell WA, 1972. Study of mutagenic effects of gum arabic (FDA No. 71-15). Unpublished. Standard Research Institute, pp. 31. Contract No. FDA 71-267.

Nie SP, Wang C, Cui SW, Wang Q, Xie MY and Phillips GO, 2013. The core carbohydrate structure of *Acacia seyal var. seyal* (Gum arabic). Food Hydrocolloids, 32, 221–227.

NTP, 1982. Carcinogenesis bioassay of gum arabic (CAS No. 9000-01-5) in F344/N rats and B6C3F1/N mice (feed study). Technical Report Series, NIH Publication No. 82-1783. Available online: http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr227.pdf

O'Dell BL, Morris ER, Pickett EE and Hogan AG, 1957. Diet composition and mineral balance in guinea pigs. Journal of Nutrition, 63, 65–77.

O'Donnell C and Baird J, 1993. Overview of gum analyses. Prepared Foods August 1993: 99–100.

Pazur JH and Li NQ, 2004. Application of antibodies for the identification of polysaccharide gum additives in processed foods. Food Additives and Contaminants, 21, 1027–1034.

Phillips GO, Ogasawara T and Ushida K, 2008. The regulatory and scientific approach to defining gum Arabic (*Acacia senegal* and *Acacia seyal*) as a dietary fibre. Food Hydrocolloids, 22, 24–35.

Prival MJ, Simmon VF and Mortelmans KE, 1991. Bacterial mutagenicity testing of 49 food ingredients gives very few positive results. Mutation Research, 26, 321–329.

Randall RC, Phillips GO and Williams PA, 1988. The role of the proteinaceous component on the emulsifying properties of gum arabic. Food Hydrocolloids, 2, 131–140.

Renard D, Lavenant-Gourgeon L, Ralet M-C and Sanchez C, 2006. Acacia Senegal gum: continuum of molecular species differing by their protein to sugar ratio, molecular weight and charges. Biomacromolecules, 7, 2637–2649.

Renard D, Garnier C, Lapp A, Schmitt C and Sanchez C, 2012. Structure of arabinogalactan-protein from Acacia gum: from porous ellipsoids to supramolecular architectures. Carbohydrate Polymers, 90, 322–332.

Renard D, Lepvrier E, Garnier C, Roblin P, Nigen M and Sanchez C, 2014. Structure of glycoproteins from Acacia gum: an assembly of ring-like glycoproteins modules. Carbohydrate Polymers, 99, 736–747.

Robbins JW and Ingledew WM, 1975. Potash guar gum-a source of halophilic bacteria. Canadian Journal of Microbiology, 22, 1658–1661.

Ross AHM, McKay LF, Busuttil A, Anderson DMW, Brydon WG and Eastwood MA, 1981. Gum arabic metabolism in the rat colon. Proceedings of the Nutrition Society, 40, 73A.

Ross AHM, Eastwood MA, Brydon WG, Anderson JR and Anderson DMW, 1983. A study of dietary gum arabic in humans. American Journal of Clinical Nutrition, 37, 368–375.

Ross AHM, Eastwood MA, Brydon WG, Busuttil A, McKay F and Anderson DMW, 1984. A study of dietary effects of gum arabic in the rat. British Journal of Nutrition, 31, 47–56.

Salyers AA, Vercellotti JR, West SHE and Wilkins TD, 1977a. Fermentation of mucins and plant polysaccharides by strains of *Bacteroides* from the human colon. Applied and Environmental Microbiology, 33, 319–322.

Salyers AA, Vercellotti JR, West SHE and Wilkins TD, 1977b. Fermentation of mucins and plant polysaccharides by strains of *Bacteroides* from the human colon. Applied and Environmental Microbiology, 33, 529–533.

Sanchez C, Schmitt C, Kolodziejczyk E, Lapp A, Gaillard C and Renard D, 2008. The acacia gum arabinogalactan fraction is a thin oblate ellipsoid: a new model based on small-angle neutron scattering and *ab initio* calculation. Biophysical Journal, 94, 629–639.

Sander I, Heimsoth MR, Wiemer K, Kespohl S, Brüning T and Merget R, 2006. Sensitization due to gum arabic (*Acacia senegal*): the cause of occupational allergic asthma or cross-reaction to carbohydrates. International Archives of Allergy and Immunology, 141, 51–56.

SCF (Scientific Committee for Food), 1991. Reports of the Scientific Committee for Food (24th Series). First Report of the Scientific Committee for Food on the essential requirements for weaning foods. Opinion expressed on 30 March 1990. Food science and techniques. Available online: http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_24.pdf

SCF (Scientific Committee on Food), 1996. Opinion on additives in nutrient preparations for use in infant formulae, follow-on formulae and weaning foods. 7 June 1996. Available online: http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_40.pdf

SCF (Scientific Committee for Food), 1998. Opinion of the Scientific Committee of Food on the applicability of the ADI (Acceptable Daily Intake) for food additives to infants. 17 September 1998. Available online: http://ec.europa.eu/food/fs/sc/scf/out13_en.html

SCF (Scientific Committee for Food), 1999. Reports of the Scientific Committee for Food (43rd Series). Opinion on certain additives for use in foods for infants and young children in good health and in foods for special medical purposes for infants and young children. Opinion expressed on 13 June 1997. Food science and techniques. Available online: http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_43.pdf

SCF (Scientific Committee on Food), 2001. Guidance on submissions for food additive evaluations by the scientific committee on food. Opinion expressed on 11 July 2001. Available online: http://ec.europa.eu/food/fs/sc/scf/out98_en.pdf

Sharma RD, 1985. Hypocholesterolemic effect of gum acacia in men. Nutrition Research, 5, 1321–1326.

Sheu CW, Cain KT, Rushbrook CJ, Jorgenson TA and Generoso WM, 1986. Test for mutagenic effect of ammoniated glycyrrhizin, butylated hydroxytoluene, and gum arabic in rodent germ cells. Environmental Mutagenesis, 8, 357–367.

Shue GM, Douglass CD and Friedman L, 1962. Nutritional studies of complex carbohydrates. Federation Proceedings, 21, 91–95.

Souw P and Rehm HJ, 1973. Untersuchungen über mikroorganismen in verdickungsmitteln. I. Mitteilung. Keimzahlen aerober mikroorganismen. Chemie, Mikrobiologie, Technologie der Lebensmittel. Food Chemistry, microbiology, technology, 2, 187–192.

Souw P and Rehm HJ, 1975a. Untersuchungen über mikroorganismen in verdickungsmitteln. II. Mitteilung. Keimzahlen anaerober sporenbildner. Chemie, Mikrobiologie, Technologie der Lebensmittel. Food Chemistry, microbiology, technology, 4, 71–74.

Souw P and Rehm HJ, 1975b. Untersuchungen über mikroorganismen in verdickungsmitteln. III. Mitteilung. Lebensdauer von Escherichia coli, Streptococcus faecalis und Staphylococcus aureus in trockenen verdickungsmitteln. Chemie, Mikrobiologie, Technologie der Lebensmittel. Food Chemistry, microbiology, technology, 4, 97–100.

Strobel S, Ferguson A and Anderson DMW, 1982. Immunogenicity of foods and food additives – *in vivo* testing of gums, arabic, karaya and tragacanth. Toxicology Letters, 14, 247–252.

Sunvold GD, Fahey GC, Merchen NR, Titgemeyer EC, Bourquin LD, Bauer LL and Reinhart GA, 1995a. Dietary fiber for dogs: IV. *In vitro* fermentation of selected fiber sources by dog fecal inoculum and in vivo digestion and metabolism of fiber-supplemented diets. Journal of Animal Sciences, 73, 1099–1109.

Sunvold GD, Fahey GC, Merchen NR, Bourquin LD, Titgemeyer EC, Bauer LL and Reinhart GA, 1995b. Dietary fiber for cats: in vitro fermentation of selected fiber sources by cat fecal inoculum and in vivo utilization of diets containing selected fiber sources and their blends. Journal of Animal Sciences, 73, 2329–2339.

TemaNord, 2002. Acacia gum. In: Food additives in Europe 2000 - Status of safety assessments of food additives presently permitted in the EU. Available online: http://www.norden.org/en/publications/publikationer/2002-560/at_download/publicationfile

Ternes W, Taeufel A, Tunger L and Zobel M, 2007. *Gummi Arabicum in Lexikon der Lebensmittel und der Lebensmittelchemie*. Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, Germany. pp. 747–748.

Thevenet F, 2010. Acacia Gum (Gum Arabic) in Food Stabilisers, Thickeners and Gelling Agents. Ed Imeson A. Blackwell Publishing Ltd, United Kingdom. pp. 11–30.

Topping DL and Clifton PM, 2001. Short-chain fatty acids and human colonic function: roles and resistant starchand nonstarch polysaccharides. Physiological Reviews, 81, 1031–1064.

Ullmann's Encyclopedia of Industrial Chemistry, 2012. Polysaccharides.

Verbeken D, Dierckx S and Dewettinck K, 2003. Exudate gums: occurrence, production, and applications. Applied Microbiology and Biotechnology, 63, 10–21.

Viinanen A, Salokannel M and Lammintausta K, 2011. Gum arabic as a cause of occupational allergy. Journal of Allergy, 2011, 1–5.

Williams PA and Phillips GO, 2009. *Handbook of hydrocolloids*. 2nd Editon, Chapter 11. Eds. Phllips GO and Williams PA (eds.). CRC Press, Inc. NW, Bocca Raton, Florida, US. pp. 228–251.

Williams PA, Phillips GO, Stephen AM and Churms SC, 2006. *Gums and Mucilages in Food Polysaccharides and Their Applications*. 2nd Edition. Taylor & Francis Group, LLC, Boca Raton, FL.

Zeiger EB, Anderson B, Haworth S, Lawlor T and Mortelmans K, 1992. *Salmonella* mutagenicity tests: V. Results from testing 311 chemicals. Environmental and Molecular Mutagenesis, 19, 2–141.

Zhang K, Wong JW, Jia Z, Vaclavikova M, Trucksess MW and Begley TH, 2014. Screening multimycotoxins in food-grade gums by stable isotope dilution and liquid chromatography/tandem mass spectrometry. Journal of AOAC International, 97, 889–895.

Abbreviations

ADI	acceptable daily intake
AFC EFSA	Former Panel on Additives, Flavourings, Processing Aids and Materials in Contact with Food
AIPG	Association for International Promotion of Gums
ANS Panel	EFSA Panel on Food Additives and Nutrient Sources added to Food
AOAC	Association of Official Agricultural Chemists

CAS	Chemical Abstracts Service
CFU	colony-forming unit
EINECS	European Inventory of Existing Commercial Chemical Substances
EMA	European Medicines Agency
FCS	Food Classification System
FDA	Food and Drug Administration
FDE	Food Drink Europe
FDRL	Food and Drug Research Laboratories
FSMP	foods for special medical purposes
GD	gestation day
GLP	Good Laboratory Practice
GNPD	Global New Products Database
HDL	high-density lipoprotein
ICGA	International Chewing Gum Association
Ig	Immunoglobulin
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	lethal dose
LDL	low-density lipoprotein
LOQ	limit of quantification
MPL	maximum permitted level
NDA	EFSA Panel on Dietetic Products, Nutrition and Allergies
NOAEL	no-observed-adverse-effect-level
OECD	Organisation for Economic Co-operation and Development
QS	quantum satis
SCF	Scientific Committee for Food
SCFA	short-chain fatty acids
SNE	specialised Nutrition Europe
TAMC	total aerobic microbial count
TLC	thin-layer chromatography
TMDI	theoretical maximum daily intake
TYMC	total combined yeasts and moulds

Appendix A – Summary of the reported use levels (mg/kg or mg/L as appropriate) of acacia gum (E 414) provided by industry

Appendix A can be found in the online version of this output ('Supporting information' section): https://doi.org/10.2903/j.efsa.2017.4741

Appendix B – Number and percentage of food products labelled with acacia gum (E 414) out of the total number of food products present in Mintel GNPD per food subcategory between 2011 and 2016

Mintel sub-category[a]	Total number of products	Products labelled with acacia	
		Number	%
Gum (chewing gum)	1,262	633	50.2
Sports Drinks	705	215	30.5
Standard & Power Mints	787	213	27.1
Non-Individually Wrapped Chocolate Pieces	4,687	960	20.5
Mixed Assortments	271	45	16.6
Liquorice	690	111	16.1
Pastilles, Gums, Jellies & Chews	3,346	315	9.4
Other Sugar Confectionery	950	84	8.8
Medicated Confectionery	891	76	8.5
Fruit/Flavoured Still Drinks	2,590	220	8.5
Energy Drinks	1,485	121	8.1
Meal Replacements & Other Drinks	990	69	7.0
Carbonated Soft Drinks	4,879	338	6.9
Snack Mixes	1,273	87	6.8
Seasonal Chocolate	4,962	305	6.1
Flavoured Alcoholic Beverages	1,800	105	5.8
Beverage Concentrates	2,097	119	5.7
Nuts	4,018	220	5.5
Marshmallows	431	19	4.4
Beverage Mixes	767	32	4.2
Spoonable Yogurt	8,752	329	3.8
Flavoured Water	1,164	37	3.2
Dairy-Based Frozen Products	7,001	201	2.9
Other Chocolate Confectionery	263	7	2.7
Lollipops	341	9	2.6
Baking Ingredients & Mixes	8,031	210	2.6
Individually Wrapped Chocolate Pieces	2,296	59	2.6
Cold Cereals	5,471	137	2.5
Boiled Sweets	858	20	2.3
Sticks, Liquids & Sprays	88	2	2.3
Snack/Cereal/Energy Bars	4,232	90	2.1
Other Frozen Desserts	1,678	35	2.1
Malt & Other Hot Beverages	921	19	2.1
Dessert Toppings	573	11	1.9
Chilled Desserts	5,584	107	1.9
Toffees, Caramels & Nougat	1,738	31	1.8
Other Snacks	117	2	1.7
Chocolate Spreads	979	16	1.6
Cakes, Pastries & Sweet Goods	11,611	186	1.6
Vegetable Snacks	511	7	1.4
Beer	7,035	94	1.3
Chocolate Tablets	7,344	91	1.2
Popcorn	981	12	1.2
Drinking Yogurt & Liquid Cultured Milk	2,886	35	1.2

Mintel sub-category[a]	Total number of products	Products labelled with acacia	
		Number	%
Other Sauces & Seasonings	851	10	1.2
Dips	1,282	15	1.2
Rice Snacks	352	4	1.1
Soft Cheese Desserts	1,364	15	1.1
Sweet Biscuits/Cookies	15,465	168	1.1
Chocolate Countlines	2,058	19	0.9
RTD (Iced) Tea	1,522	14	0.9
Shelf-Stable Desserts	2,950	25	0.8
Wheat & Other Grain-Based Snacks	1,664	14	0.8
Instant Rice	120	1	0.8
Cream	1,454	12	0.8
Fruit Snacks	2,892	23	0.8
Rice/Nut/Grain & Seed Based Drinks	954	7	0.7
Meal Kits	1,809	13	0.7
Dressings & Vinegar	3,035	19	0.6
Nectars	3,581	22	0.6
Meat Substitutes	1,908	11	0.6
Processed Cheese	1,875	9	0.5
Pizzas	3,886	18	0.5
Caramel & Cream Spreads	243	1	0.4
Sucrose	975	4	0.4
Artificial Sweeteners	269	1	0.4
Liqueur	1,467	5	0.3
Potato Snacks	4,388	13	0.3
Coffee	6,749	18	0.3
Syrups	408	1	0.2
Poultry Products	5,483	13	0.2
Baby Fruit Products, Desserts & Yogurts	1,405	3	0.2
Savoury Vegetable Pastes/Spreads	1,416	3	0.2
Fresh Cheese & Cream Cheese	2,457	5	0.2
Prepared Meals	9,894	20	0.2
Hot Cereals	1,021	2	0.2
Instant Pasta	549	1	0.2
Cooking Sauces	4,446	8	0.2
Sandwiches/Wraps	2,406	4	0.2
Soy Based Drinks	609	1	0.2
Confiture & Fruit Spreads	4,266	7	0.2
Stocks	1,233	2	0.2
Nut Spreads	645	1	0.2
Corn-Based Snacks	1,955	3	0.2
Meat Pastes & Pates	2,776	4	0.1
Hors d'oeuvres/Canapes	3,631	5	0.1
Dry Soup	1,466	2	0.1
RTD (Iced) Coffee	768	1	0.1
Salads	2,337	3	0.1
Mayonnaise	802	1	0.1
Cider	837	1	0.1

Mintel sub-category[a]	Total number of products	Products labelled with acacia	
		Number	%
Pastry Dishes	1,721	2	0.1
Margarine & Other Blends	889	1	0.1
Wine	3,590	4	0.1
Sandwich Fillers/Spreads	901	1	0.1
Rice	2,932	3	0.1
Fish Products	10,920	11	0.1
Instant Noodles	995	1	0.1
Stuffing, Polenta & Other Side Dishes	1,999	2	0.1
Water-Based Frozen Desserts	1,072	1	0.1
Vegetables	9,286	8	0.1
Fruit	2,448	2	0.1
Meat Products	13,984	11	0.1
Flavoured Milk	1,272	1	0.1
Bread & Bread Products	8,946	7	0.1
Tea	7,889	6	0.1
Table Sauces	5,376	4	0.1
Savoury Biscuits/Crackers	4,219	3	0.1
Soft Cheese & Semi-Soft Cheese	4,995	3	0.1
Pasta Sauces	3,398	2	0.1
Wet Soup	3,751	2	0.1
Seasonings	8,423	3	0.0
Potato Products	2,870	1	0.0
Pasta	8,872	1	0.0
Total sample	**384,088**	**6,666**	**1.7(b)**

(a): According to the Mintel GNPD food categorisation.
(b): In total, around 1.7% of the foods available on the Mintel GNPD are labelled with acacia gum (E 414) between 2011 and 2016.

Appendix C – Concentration levels of acacia gum (E 414) used in the refined exposure scenarios (mg/kg or mL/kg as appropriate)

Appendix C can be found in the online version of this output ('Supporting information' section): https://doi.org/10.2903/j.efsa.2017.4741

Appendix D – Summary of total estimated exposure of acacia gum (E 414) from their use as food additives for the maximum level exposure scenario and the refined exposure assessment scenarios per population group and survey: mean and high level (mg/kg bw per day)

Appendix D can be found in the online version of this output ('Supporting information' section): https://doi.org/10.2903/j.efsa.2017.4741

WHO Technical Report Series

1007

Evaluation of certain food additives

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

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. An annual subscription to this series, comprising about four to six such reports, costs CHF 150.00 / US$ 180.00 (CHF 105.00/US$ 126.00 in developing countries). For further information, 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; order online: http://www.who.int/bookorders).

WHO Technical Report Series
1007

Evaluation of certain food additives

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

This report contains the collective views of an international groups 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-fourth report of the Joint FAO/WHO Expert Committee on Food Additives

(WHO technical report series ; no. 1007)

Subject headings are available from WHO institutional repository

ISBN 978-92-4-121016-4
ISSN 0512-3054

Design: Rania Spatha (http://www.raniaspatha.com)

Printed in Malta

Contents

Annex 3

List of participants

Eighty-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives
Rome, 6–15 June 2017

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, Surbiton, London, England, United Kingdom

Dr R. Cantrill, American Oil Chemists' Society (AOCS), Urbana, Illinois, USA (*Chairperson*)

Dr E. Dessipri, General Chemical State Laboratory, Athens, Greece

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

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*)

Dr A. Mattia, Senior Science and Policy Staff, Office of Food Additive Safety, Center for Food Safety and Applied Nutrition, United States Food and Drug Administration, College Park, Maryland, USA (*Vice-Chairperson*)

Dr U. Mueller, Australian Pesticides and Veterinary Medicines Authority (APVMA), Kingston, 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, Precision Scientific Laboratories, Dubai, United Arab Emirates

Dr H.J. Yoon, Food Standard Division, Ministry of Food and Drug Safety, Seoul, Republic of Korea

Secretariat

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 P. Boon, Department Food Safety, Centre for Nutrition, Prevention and Health Services, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands (*WHO Temporary Adviser*)

Ms A. Bruno, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization of the United Nations, Rome, Italy (*Codex Secretariat*)

Dr M. Choi, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization of the United NationsUN, Rome, Italy (*Codex Secretariat*)

Dr L. DeJager, Division of Analytical Chemistry, Office of Regulatory Science, Center for Food Safety & Applied Nutrition, United States Food and Drug Administration, College Park, Maryland, USA (*FAO Expert*)

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 Vice-Chairperson*)

Dr V. Fattori, Food Safety and Quality Unit, Agriculture and Consumer Protection Department, FAO, Rome, Italy (*FAO Secretariat*)

Dr R. Gürtler, Federal Institute for Risk Assessment (BfR), Department Food Safety, Unit Food Toxicology, Berlin, Germany (*WHO Temporary Adviser*)

Dr H. Hallstrom, Risk and Benefit Assessment Department, National Food Agency, Uppsala, Sweden (*WHO Temporary Adviser*)[1]

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 C. Lambré, Dammartin en Goële, France (*WHO Temporary Adviser*)

Dr K. Laurvick, United States Pharmacopeial Convention, Rockville, Maryland, USA (*FAO Expert*)

Dr J.C. Leblanc, Food Safety and Quality Unit, Agriculture and Consumer Protection Department, Food and Agriculture Organization of the United Nations, Rome, Italy (*FAO Secretariat*)

Dr M. Lipp, Agriculture and Consumer Protection Department, Food and Agriculture Organization of the United Nations, Rome, Italy (*FAO Joint Secretary*)

[1] Unable to attend the meeting.

Dr K. Muldoon Jacobs, United States Pharmacopeial Convention, Rockville, Maryland, USA (*WHO Temporary Adviser*)

Ms C. Mulholland, Chemical Risk Assessment Unit, Food Standards Agency, London, United Kingdom (*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*)

Ms M. Sheffer, Orleans, Ontario, Canada (*WHO Technical Editor and Co-rapporteur*)

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*)

Dr A. Tada, Division of Food Additives, National Institute of Health Science, Tokyo, Japan (*FAO Expert*)

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*)

Ms R. Yamamoto, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization of the United Nations, Rome, Italy (*Codex Secretariat*)

Dr X. Yang, Guangdong Provincial Center for Disease Control and Prevention, 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*)

List of abbreviations

ADI	acceptable daily intake
bw	body weight
CAS	Chemical Abstracts Service
CCFA	Codex Committee on Food Additives
CCFA49	Forty-ninth Session of the Codex Committee on Food Additives
CIFOCOss	FAO/WHO Chronic individual food consumption database – Summary statistics
CITREM	citric and fatty acid esters of glycerol
CYP	cytochrome P450
CSAF	chemical-specific adjustment factor
EFSA	European Food Safety Authority
F_0	parental generation
F_1	first filial generation
FAO	Food and Agriculture Organization of the United Nations
FSANZ	Food Standards Australia New Zealand
GEMS/Food	Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme
GLP	good laboratory practice
GSFA	(Codex) General Standard for Food Additives
HPLC	high-performance liquid chromatography
HPLC–UV	high-performance liquid chromatography with ultraviolet detection
IC_{50}	half maximal inhibitory concentration
INS	International Numbering System for Food Additives
IPCS	International Programme on Chemical Safety
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	median lethal dose
NOAEL	no-observed-adverse-effect level
no./No.	number
OECD	Organisation for Economic Co-operation and Development
Panx1	pannexin 1
UV	ultraviolet
USA	United States of America
USFDA	United States Food and Drug Administration
WHO	World Health Organization

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

Evaluation of certain food additives. WHO Food Additives Series, No. 75, 2018.

Specifications are issued separately by FAO under the title:

Compendium of food additive specifications. FAO JECFA Monographs 20, 2018.

1. Introduction

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) met in Rome from 6 to 15 June 2017. The meeting was opened on behalf of Director-General Graciano da Silva of the Food and Agriculture Organization of the United Nations (FAO) by Dr Ren Wang, Assistant Director-General, FAO Agriculture and Consumer Protection Department.

Dr Wang preceded his opening remarks by welcoming Dr Yongxiang Fan, Vice-Chairperson of the Codex Committee on Food Additives, and all other meeting participants. Summarizing the mandate of the Codex Alimentarius Commission as simultaneously protecting the health of consumers, ensuring fair practices in the food trade and promoting coordination of all food standards work undertaken by international governmental and nongovernmental organizations, Dr Wang reminded the meeting that Codex standards are supported by scientifically sound, globally relevant yet independent safety assessments provided by experts and specialists in a wide range of disciplines. Noting that the FAO and WHO advisory bodies provide a focal point for food-related safety assessments through a number of joint expert committees for a large variety of food safety topics including food additives – the topic of this JECFA meeting – Dr Wang emphasized that participants had been invited not as representatives of their employer or country, but to provide sound and independent scientific advice to generate food standards designed to be health-protective for all consumers and trade-inclusive for all regions and countries.

Dr Wang pointed out that several people at the meeting had also been present in 2016, for JECFA's 60th anniversary, as well as in preceding years, thus contributing their continued guidance and providing the necessary stability. Dr Wang welcomed them as well as participants present at JECFA for their first time. He emphasized that, as the sciences evolve, JECFA needs everyone to add to the pool of knowledge, helping to update processes, procedures and approaches to incorporate new scientific insights and consider more data and more diverse studies and inputs in JECFA deliberations.

1.1 Declarations of interests

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

2. General considerations

As a result of the recommendations of the first Joint FAO/WHO Conference on Food Additives, held in September 1955 *(1)*, there have been 83 previous meetings of the Committee (Annex 1). The present meeting was convened on the basis of a recommendation made at the eighty-second meeting (Annex 1, reference *230*). The tasks before the Committee were to:

- elaborate further principles for evaluating the safety of food additives (section 2);
- review and prepare specifications for certain food additives (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 Forty-ninth Session of the Codex Committee on Food Additives (CCFA)

Dr Yongxiang Fan, Vice-Chair of CCFA, provided the Committee with an update on the work of CCFA since the eighty-second meeting of JECFA (Annex 1, reference *230*).

The Forty-ninth Session of the CCFA (CCFA49) noted the conclusions of the eighty-second meeting of JECFA on the safety of 12 substances *(2)*. CCFA49 agreed to include lutein esters from *Tagetes erecta* (International Numbering System for Food Additives [INS] No. 161b(iii)) and octenyl succinic acid–modified gum arabic (INS No. 423) in Table 3 ("Additives Permitted for Use in Food in General, Unless Otherwise Specified, in Accordance with Good Manufacturing Practice") of the *Codex General Standard for Food Additives* (GSFA) (CODEX STAN 192–1995) (*3*). CCFA49 solicited members to provide more information or data to JECFA to complete the evaluation for carob bean gum (INS No. 410) and cassia gum (INS No. 427) and noted that no action was necessary for other substances.

CCFA49 finalized work on more than 400 provisions of the GSFA and forwarded specifications for the identity and purity of 15 food additives (one new specification and 14 revised specifications) and 29 flavourings (23 new specifications and six revised specifications) prepared by the eighty-second meeting of JECFA and recommended to the Fortieth Session of the Codex Alimentarius Commission for adoption. CCFA49 agreed to amend the introduction of the *List of Codex Specifications for Food Additives* (http://www.codexalimentarius.net/gsfaonline/foods/index.html) to address the concerns on the reference made to secondary additives in the specifications. In addition to adding functional classes and technological purposes to two food additives,

new INS numbers were also assigned to five food additives. CCFA49 agreed on a revised priority list of substances for evaluation (or re-evaluation) by JECFA, which includes 62 substances and 70 flavourings. CCFA49 also agreed to remove the asterisked (*) note indicating those substances high on the CCFA priority list, as the working group had not discussed this matter.

CCFA49 also established an electronic working group that will address the concerns for the use of nitrates and nitrites as food additives. The working group will analyse which issues CCFA can address and clarify the scope of the question(s) that JECFA or another appropriate FAO/WHO scientific advice body can address by taking into consideration the feasibility and data availability for such advice.

CCFA49 continued work on aligning food additive provisions in the Codex standards and the corresponding provisions of the GSFA. CCFA49 agreed that the Chairs of the working groups on GSFA, alignment, INS and JECFA priority, working with China (the host of CCFA), develop a discussion paper on future strategies for CCFA, to be considered at the Fiftieth Session of the CCFA. This paper will also consider aspects of prioritization of substances on the priority list that relate to JECFA evaluation.

2.2 Principles governing the toxicological evaluation of compounds on the agenda

In making recommendations on the safety of food additives, the Committee took into consideration the principles established and contained in the 2009 publication *Environmental Health Criteria 240: Principles and methods for the risk assessment of chemicals in food* (EHC 240)*(4)*.

2.2.1 Information requirements for submissions on products derived from natural sources

The Committee noted that, at the current meeting, a number of food additives were evaluated that were derived from natural sources. The Committee recalled that at previous meetings the need for sponsors to provide sufficient data for chemical, technical, dietary exposure and toxicological evaluation was stressed. At its thirty-first meeting, the Committee emphasized that "A full understanding of the source and chemical nature of such products was considered essential for an evaluation of their safety-in-use" (Annex 1, reference *77*). At the sixty-eighth meeting, the Committee provided considerations on "Extensions of an existing ADI to substances obtained from different sources and/or by different manufacturing processes" (Annex 1, reference *187*).

The Committee recognized that a component of interest (e.g. carotenes) may be present in the product of commerce at a low percentage relative to other components either because it is extracted together with components of similar polarity or solubility or because of subsequent standardization in the final product formulation. The Committee also recognized that some substances (e.g. gums or tannins) are complex mixtures and their components are affected to varying degrees, depending on their source or through processing. It is important to fully characterize all components of the final product, taking care to also provide the detailed manufacturing process as well as information on the carryover of substances from the starting material to the final product.

The present Committee again stressed that a full characterization of the products in commerce and a relevant set of biochemical and toxicological data on such products are essential for the Committee to develop a specifications monograph and the related safety assessment. It is not possible to complete the evaluation of a food additive if its composition cannot be compared to the substances tested biochemically and toxicologically. This is particularly important where the submission relies on literature data.

The Committee encourages CCFA to consider the above information requirements before accepting proposals for food additive evaluations to be included in the CCFA priority list.

2.2.2 Update on activities relevant to JECFA

The Committee was provided with an update of work in the WHO International Programme on Chemical Safety (IPCS). The WHO Chemical Risk Assessment Network and its activities were described, including the work on a review of how chemical-specific adjustment factors (CSAFs) are being used in regulatory and non-regulatory risk assessments.

The Secretariat informed the Committee about ongoing activities on risk assessment methodology and update of certain chapters of EHC 240: *Principles and methods for the risk assessment of chemicals in food (4)*. In particular, more detailed guidance on the interpretation and evaluation of genotoxicity studies will be developed; as well, the guidance on dose–response modelling and application of the benchmark dose approach will be updated. The chapter on exposure assessment will be updated, taking all recent developments into account. Further guidance will also be developed on the evaluation of enzyme preparations.

The Committee was also informed that the JECFA guidance for setting acute reference doses for veterinary drugs is now available online: http://www.who.int/foodsafety/chem/jecfa/Guidance-document-ARfD-2017.pdf?ua=1.

WHO recently published a distance learning tool on how to access and analyse the food contamination data submitted to the Global Environment Monitoring System – Food Contamination Monitoring and Assessment

Programme (GEMS/Food) database. This tool was developed in collaboration with the Chulabhorn Research Institute (Bangkok, Thailand), a WHO Collaborating Centre. A password-protected access to the learning tool is available upon request from: vergerp@who.int.

2.3 Food additive specifications and analytical methods

2.3.1 Corrigenda for specifications monographs

The following requests for corrections in JECFA Food Additives Specifications Monographs were received by the JECFA Secretariat (see Table 1). The Committee at the current meeting evaluated the information provided and made the following corrections. These corrections will be published in the electronic versions and in the online database of JECFA Food Additives Specifications Monographs. The information is provided here to make interested parties aware of these changes.

Table 1
Corrections in JECFA Food Additives Specifications Monographs

Food additive	Original text	New text	Additional explanations
Carob bean gum (clarified) (JECFA 82, FAO JECFA Monographs 19, 2016)	Heading: Carob bean gum	Heading: Carob bean gum (clarified)	In the original publication of FAO JECFA Monographs 19, the monograph heading omitted ("(clarified)"), while the specifications referred to the clarified carob bean gum
Carob bean gum (JECFA 82, FAO JECFA Monographs 19, 2016)	None Specifications have been prepared and adopted at JECFA 82 for carob bean gum but were not published in the FAO JECFA Monographs 19.	Please refer to http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/detail/en/c/484/	
CITREM (JECFA 82, FAO JECFA Monographs 19, 2016)	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).	Lead (Vol. 4) Not more than 2 mg/kg. (Not more than **0.5** mg/kg for use in infant formula and formula for special medical purposes intended for infants).	Transcription error
Diammonium hydrogen phosphate (JECFA 59, FAO JECFA Monographs 1, 2006)	CAS 7783-54-0	CAS 7783-28-0	
Dimethyl dicarbonate (JECFA 63, FAO JECFA Monographs 1, 2006)	CAS 004-525-33-1	CAS 4525-33-1	
Ferrous sulfate (JECFA 53, FAO JECFA Monographs 1, 2006)	CAS 7720-78-7	CAS 7782-63-0	
Ferrous sulfate, dried (JECFA 53, FAO JECFA Monographs 1, 2006)	No CAS number	CAS 7720-78-7	
Paprika extract (JECFA 79, FAO JECFA Monographs 16, 2014)	Preamble: An ADI of 0–1.5 mg/kg bw was allocated at the 79th JECFA (2014).	Preamble: An ADI of 0–1.5 mg/kg bw **(expressed as total carotenoids)** was allocated at the 79th JECFA (2014).	
Paprika oleoresin (JECFA 59, FAO JECFA Monographs 1, 2006)	INS160c	INS160c(i)	
L-Malic acid (flavouring)	Optical rotation: −0.23 (25 °C)	Optical rotation: −2.3 (8.5 g/100 mL water at 20 °C)	The magnitude and direction of the optical rotation are dependent on solvent, temperature and concentration of L-malic acid.

ADI: acceptable daily intake; bw: body weight; CAS: Chemical Abstracts Service; CITREM: citric and fatty acid esters of glycerol; FAO: Food and Agriculture Organization of the United Nations; JECFA: Joint FAO/WHO Expert Committee on Food Additives
Bolding for clarity only.

3. Specific food additives

The Committee evaluated the safety of nine food additives and revised the specifications for five other food additives. Information on the safety evaluations and specifications is summarized in Annex 2.

3.1 Safety evaluations[1]

3.1.1 Brilliant Blue FCF

Explanation

Brilliant Blue FCF (Chemical Abstracts Service [CAS] No. 3844-45-9; INS No. 133) is a dye with a triphenylmethane base structure permitted as a food colour in the European Union, Japan, the United States of America (USA) and other regions. It is used for colouring breakfast cereals, cakes and cupcakes, candies, chewing gum, dairy products, decorations for baking, flavoured water and frozen treats.

The Committee previously evaluated the use of Brilliant Blue FCF as a food colour at the thirteenth meeting in 1969 (Annex 1, reference *19*). The specifications for Brilliant Blue FCF were prepared at the twenty-eighth JECFA meeting in 1984 and revised for metal specifications at the fifty-ninth meeting in 2002 (Annex 1, references *66* and *160*). An acceptable daily intake (ADI) of 0–12.5 mg/kg body weight (bw) was established by the Committee in 1969 (Annex 1, reference *19*). The ADI was based on a no-observed-adverse-effect level (NOAEL) of 5% (equivalent to 2500 mg/kg bw per day) derived from a chronic dietary toxicity study in rats (Hansen et al., 1966), with no explanation for the 200-fold uncertainty factor. More recent studies, including studies on absorption and excretion, biochemical effects, long- and short-term toxicity, carcinogenicity, genotoxicity, reproductive and developmental toxicity and allergenicity as well as studies on neurobehavioural effects and interaction with the membrane protein pannexin 1 (Panx1), have since become available.

Brilliant Blue FCF has been evaluated by the present Committee at the request of the Forty-eighth Session of the CCFA (FAO/WHO, 2016). Almost all of the new data were provided by the sponsor. Only a few additional publications were identified in a literature search. The pre-1969 studies described below were considered by the Committee at the thirteenth meeting in 1969 (Annex 1, reference *19*).

Chemical and technical considerations

Brilliant Blue FCF consists mainly of disodium 3-[*N*-ethyl-*N*-[4-[[4-[*N*-ethyl-*N*-(3-sulfobenzyl)amino]phenyl](2-sulfophenyl)methylene]-2,5-cyclohexadiene-

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

1-ylidene]ammoniomethyl]benzenesulfonate and its isomers, together with subsidiary colouring matters, as well as sodium chloride and/or sodium sulfate as the principal uncoloured components. It is manufactured by condensing 2-formylbenzenesulfonic acid with a mixture of 3-[(*N*-ethyl-*N*-phenylamino) methyl]benzenesulfonic acid and its 2- and 4-isomers to form the leuco base precursor. Oxidation of the leuco base precursor with either chromium- or manganese-containing compounds produces the dye, which is isolated as the disodium salt. The dye contains not less than 85% total colouring matters. Impurities include unreacted starting material and reaction by-products (~2%), subsidiary colouring matters (≤6%), residual leuco base precursor (≤5%), unsulfonated primary aromatic amines (≤0.01% calculated as aniline), lead (≤2 mg/kg), chromium (≤50 mg/kg) and manganese (≤100 mg/kg).

Biochemical aspects

When Brilliant Blue FCF was administered orally to rats, almost the entire dose was excreted unchanged in the faeces within 40 hours. The colour was also found in the bile of rats, rabbits and dogs after oral administration. Only 5% of the dose administered was excreted in the bile of dogs (Hess & Fitzhugh, 1953, 1954, 1955). In other studies, absorption of Brilliant Blue FCF was about 0.5% in rats (Brown et al., 1980; Phillips et al., 1980), with more than 99% of total intake excreted in the faeces and less than 1% recovered in the urine. Results of thin-layer chromatography (TLC) of urine and bile samples 24 hours after ingestion showed that about 95% of excreted radioactivity was unaltered ^{14}C-radiolabelled Brilliant Blue FCF and that about 5% was unidentified metabolite(s) or degradation product(s). Mass spectrometric analysis was, however, not used.

An ex vivo porcine tongue system showed that about 0.2% of Brilliant Blue FCF diffused through the surface oral mucosa layers (Lucová et al., 2013).

Equilibrium dialysis methods have demonstrated that Brilliant Blue FCF binds to rat plasma protein (Iga, Awazu & Nogami, 1971; Iga et al., 1971). The extent of binding of Brilliant Blue FCF with plasma protein was 65% after 160 hours of dialysis at 37 °C.

In an in vitro study in which the Xenopus oocyte expression system was used for pharmacological investigations on purinergic P2 receptors that interact with the membrane channel protein Panx1 in inflammasome signalling, Brilliant Blue FCF was shown to be a selective inhibitor of Panx1 channels, with a half maximal inhibitory concentration (IC_{50}) of 0.27 µmol/L; no significant effect on the P2X7R receptor was observed at concentrations as high as 100 µmol/L (Wang, Jackson & Dahl, 2013). The Committee was aware that Panx1 activation/inhibition is one of several signalling pathways involved in various physiological processes at the cellular level (e.g. immune function) and that there are many exogenous and endogenous modulators of these pathways. Interactions of

substances with P2 receptors and Panx1 are an active area of research, particularly in development of drug treatments for diverse chronic diseases. The Committee noted that a similar pattern of channel inhibition was observed with Fast Green FCF, and further research may clarify if the inhibition of Panx1 observed in an in vitro system has any relevance for the safety assessment for substances in food.

Toxicological studies

The acute toxicity of Brilliant Blue FCF is low. The median lethal dose (LD_{50}) in mice (Sasaki et al., 2002) and rats (Lu & Lavallee, 1964) was higher than 2000 mg/kg bw.

In a 1-year dietary study, 12 dogs were fed Brilliant Blue FCF (purity not reported) at 0%, 1% or 2%. No clinical signs, gross lesions or microscopic pathological findings were attributed to exposure to Brilliant Blue FCF (Hansen et al., 1966).

The long-term toxicity of Brilliant Blue FCF was investigated in three studies in mice and five in rats.

No evidence of treatment-related carcinogenicity was found when male and female mice were fed Brilliant Blue FCF at a dose of 1 mg/kg bw per day over 500–700 days (Waterman & Lignac, 1958).

The administration of Brilliant Blue FCF to male and female mice for up to 80 weeks in the diet at concentrations of 0%, 0.015%, 0.15% or 1.5% (equivalent to 0, 20, 200 and 2000 mg/kg bw per day, respectively) resulted in slight reduction in weight gain and increased incidence of foam cells in the liver at the highest dose (Rowland et al., 1975). The NOAEL was 0.15% (equivalent to 200 mg/kg bw per day).

In a long-term toxicity study in which Brilliant Blue FCF was fed to male and female mice for 24 months (104 weeks) at dietary concentrations of 0%, 0.5%, 1.5% or 5% (equal to 0, 661, 2064 and 7354 mg/kg bw per day for males and 0, 819, 2562 and 8966 mg/kg bw per day for females, respectively), the NOAEL was 5% (equal to 7354 mg/kg bw per day), the highest concentration tested (IRDC, 1981a; Borzelleca, Depukat & Hallagan, 1990). The Committee noted that the survival at the end of the study was about 50% in both control and treated groups.

When Brilliant Blue FCF was fed to male and female rats at a dietary level of 4% for 600 days, there were no treatment-related tumours (Willheim & Ivy, 1953). In another long-term toxicity study in which rats were fed a diet containing 0.1% Brilliant Blue FCF over their lifetime (daily intake 10–15 mg), no treatment-related tumours were found (Klinke, 1955). Similarly, when male and female rats were fed diets containing 0%, 0.3% or 3% Brilliant Blue FCF for 75 weeks, no treatment-related adverse effects were observed on tumour incidence, growth or haematological findings (Mannell, Grice & Allmark, 1962).

In its previous evaluation at the thirteenth meeting (Annex 1, reference *19*), the Committee established an ADI of 0–12.5 mg/kg bw based on a 2-year toxicity study in which male and female rats were fed a diet containing 0%, 0.5%, 1.0%, 2.0% or 5.0% Brilliant Blue FCF. The NOAEL was 5.0% (equivalent to 2500 mg/kg bw per day), the highest concentration tested (Hansen et al., 1966).

In a long-term toxicity study that included an in utero exposure phase, Brilliant Blue FCF was fed to the F_0 rats for up to 17 weeks at levels of 0%, 0.1%, 1% or 2% (calculated to provide doses of 0, 50, 514 and 1073 mg/kg bw per day for males and 0, 62, 631 and 1318 mg/kg bw per day for females, respectively). The F_1 animals were administered Brilliant Blue FCF at the same dose levels for up to 116 weeks for males and 111 weeks for females. The NOAEL was 1% (equal to 631 mg/kg bw per day), based on 15% decreased mean terminal body weight and decreased survival of female rats at the highest dose level (IRDC, 1981b; Borzelleca, Depukat & Hallagan, 1990).

The Committee concluded from these studies in mice and rats that there is no concern with respect to carcinogenicity of Brilliant Blue FCF.

No mutagenic activity has been observed with Brilliant Blue FCF in several in vitro mutagenicity studies conducted in *Salmonella typhimurium*, *Bacillus subtilis* and *Escherichia coli*. Positive findings were reported in two in vitro chromosomal aberration assays, one in vitro micronucleus assay and one in vitro comet assay in mammalian cells, but these studies had a number of shortcomings (Kawachi et al., 1980; Ishidate et al., 1984; Kus & Eroglu, 2015; Pandir, 2016). In contrast, negative results were obtained in an in vivo micronucleus assay in bone marrow (Hayashi et al., 1988) and a comet assay in the stomach, colon, liver, kidney, bladder, lung, brain and bone marrow of mice (Sasaki et al., 2002). Based on the available data, the Committee concluded that there is no concern with respect to genotoxicity of Brilliant Blue FCF.

No treatment-related adverse reproductive effects were found in a single-generation study in male and female rats fed Brilliant Blue FCF at doses up to 1318 or 1073 mg/kg bw per day, respectively (IRDC, 1981b; Borzelleca, Depukat & Hallagan, 1990). Similarly, no treatment-related adverse effects were seen in a three-generation study in rats treated with Brilliant Blue FCF at doses up to 1000 mg/kg bw per day (BioDynamics Inc., 1971). In developmental toxicity studies, no adverse effects were reported in rats treated with Brilliant Blue FCF at doses up to 2000 mg/kg bw per day (BioDynamics Inc., 1972a) or in rabbits at doses up to 200 mg/kg bw per day (BioDynamics Inc., 1972b).

Other studies have reported no evidence for allergenicity (Kreindler, Slutsky & Haddad, 1980), skin irritation (BIBRA, 1990), dermal sensitization (BIBRA, 1990) or skin cancer (Carson, 1984) as a result of treatment with Brilliant Blue FCF.

In a one-generation study on neurobehavioural development in mice (Tanaka et al., 2012), Brilliant Blue FCF was given in the diet at concentrations of 0%, 0.08%, 0.24% or 0.72% (equal to 0, 111–407, 347–1287 and 1032–3856 mg/kg bw per day, respectively, exposure depending on gestational age). The high dose of Brilliant Blue FCF resulted in a few statistically significant effects on neurobehavioural development (exploratory behaviour and surface righting response). However, the Committee noted that the effects on exploratory behaviour were inconsistent and that there were no effects from exposure to Brilliant Blue at any dose in several other neurobehavioural tests in this study. The Committee concluded that the findings were not robust enough to be used in the safety assessment.

Observations in humans

Case reports describe the use of Brilliant Blue FCF in enteral feeding solutions associated with discoloration of skin, urine and serum and toxicity, including 12 deaths (WHO, 2003; Maloney & Brand, 2016). The Committee noted that these case reports relate to seriously ill patients, particularly those with increased gut permeability (e.g. patients with sepsis), and that a causal relationship with Brilliant Blue FCF has not been established.

Assessment of dietary exposure

Estimates of dietary exposure to Brilliant Blue FCF published by the European Food Safety Authority (EFSA) (EFSA, 2010), Food Standards Australia New Zealand (FSANZ) (FSANZ, 2012), the United States Food and Drug Administration (USFDA) (Doell et al., 2016), India (Dixit et al., 2011), Kuwait (Husain et al., 2006) and the Republic of Korea (Ha et al., 2013) were available to the Committee. The estimate of dietary exposure to Brilliant Blue FCF calculated by EFSA (4.8 mg/kg bw per day for children at the 95th percentile) was much higher than those of the USFDA and FSANZ (both 0.2 mg/kg bw per day for children at the 90th percentile) and the Republic of Korea (0.03 mg/kg bw per day for the whole population at the 95th percentile). Estimates from India and Kuwait were also lower than the EFSA estimates, but higher than the estimates from the USFDA and FSANZ. The Committee considered that the higher values in the EFSA estimates were due to the use of maximum reported use levels, whereas the other studies used mean analysed levels. The Committee concluded that the use of the more conservative EFSA estimate of 5 mg/kg bw per day should be considered in the safety assessment for Brilliant Blue FCF.

Evaluation

The Committee concluded that the available data support the revision of the ADI for Brilliant Blue FCF and that the study on long-term toxicity in rats should be

considered as the pivotal study (IRDC, 1981b; Borzelleca, Depukat & Hallagan, 1990). In this study, a NOAEL of 631 mg/kg bw per day was identified, based on a 15% decrease in mean terminal body weight and decreased survival of females at 1318 mg/kg bw per day. The Committee established an ADI of 0–6 mg/kg bw based on this NOAEL by applying an uncertainty factor of 100 for interspecies and intraspecies differences.

The Committee noted that the conservative dietary exposure estimate of 5 mg/kg bw per day (95th percentile for children) is less than the upper limit of the ADI of 0–6 mg/kg bw established for Brilliant Blue FCF and concluded that dietary exposure to Brilliant Blue FCF for children and all other age groups does not present a health concern.

The previous ADI of 0–12.5 mg/kg bw was withdrawn.

A toxicological and dietary exposure monograph was prepared.

At the present meeting, the existing specifications for Brilliant Blue FCF were revised, and a maximum limit for manganese was added. High-performance liquid chromatography (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 or aqueous ammonium acetate.

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

References

1. BIBRA (1990). Toxicity profile of Brilliant Blue FCF. BIBRA Toxicology International, Wallington, Surrey, United Kingdom. 2nd Edition 1990 by BIBRA. Surrey, United Kingdom. Submitted by the International Association of Color Manufacturers (IACM) to WHO on 30 November 2016.
2. BioDynamics Inc. (1971). A three-generation reproduction study of FD&C Blue 1 in rats. Project no. 71R-738. BioDynamics Inc., East Millstone, NY, USA. Unpublished study report submitted by the International Association of Color Manufacturers (IACM) to WHO on 30 November 2016.
3. BioDynamics Inc. (1972a). FD&C Blue #1 Segment II. Rabbit teratology study. Project no. 71R-721C. BioDynamics Inc., East Millstone, NY, USA. Unpublished study report submitted by the International Association of Color Manufacturers (IACM) to WHO on 30 November 2016.
4. BioDynamics Inc. (1972b). FD&C Blue #1 Segment II. Rat teratology study. Project no. 71R-719C. BioDynamics Inc., East Millstone, N. Y., USA. Unpublished study report submitted by the International Association of Color Manufacturers (IACM) to WHO on 30 November 2016.
5. Borzelleca JF, Depukat K, Hallagan JB (1990). Lifetime toxicity/carcinogenicity studies of FD & C Blue No. 1 (brilliant blue FCF) in rats and mice. Food Chem Toxicol. 28(4):221–34.
6. Brown JP, Dorsky A, Enderlin FE, Hale RL, Wright VA, Parkinson TM (1980). Synthesis of ^{14}C-labelled FD & C Blue No. 1 (Brilliant Blue FCF) and its intestinal absorption and metabolic fate in rats. Food Cosmet Toxicol. 18(1):1–5.
7. Carson S (1984). Skin painting studies in mice with 14 FD&C and D&C Colors: FD&C Blue no. 1, Red no. 3, and Yellow no. 5, D&C Red no. 7, Red no. 9, Red no. 10, Red no. 19, Red no. 21, Red no. 27, Red no. 31, Red no. 36, Orange no. 5, Orange no. 10, and Orange no. 17. J Toxicol Cutaneous Ocul Toxicol. 3(4):357–70.
8. Dixit S, Purshottam SK, Khanna SK, Das M (2011). Usage pattern of synthetic food colours in different states of India and

exposure assessment through commodities preferentially consumed by children. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 28(8):996–1005.
9. Doell DL, Folmer DE, Lee HS, Butts KM, Carberry SE (2016). Exposure estimate for FD&C colour additives for the US population. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 33(5):782–97. doi:org/10.1080/19440049.2016.1179536.
10. EFSA (2010). Scientific opinion on the re-evaluation of Brilliant Blue FCF (E 133) as a food additive. EFSA J. 8(11):1853. doi:org/10.2903/j.efsa.2010.1853.
11. FAO/WHO (2016). 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 Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission (REP16/FA).
12. FSANZ (2012). Supplementary report to the 2008 Survey of added colours in foods available in Australia. Canberra: Food Standards Australia New Zealand.
13. Ha MS, Ha SD, Choi SH, Bae DH (2013). Exposure assessment of synthetic colours approved in Korea. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 30(4):643–53. doi:org/10.1080/19440049.2013.768358.
14. Hansen WH, Fitzhugh OG, Nelson AA, Davis KJ (1966). Chronic toxicity of two food colors, brilliant blue FCF and indigotine. Toxicol Appl Pharmacol. 8(1):29–36.
15. Hayashi M, Kishi M, Sofuni T, Ishidate M Jr (1988). Micronucleus tests in mice on 39 food additives and eight miscellaneous chemicals. Food Chem Toxicol. 26(6):487–500.
16. Hess SM, Fitzhugh OG (1953). Metabolism of coal-tar dyes. I. Triphenylmethane dyes (Abstract No. 1090). Fed Proc. 12:330–1.
17. Hess SM, Fitzhugh OG (1954). Metabolism of coal-tar colours. II. Bile studies (Abstract No. 1201). Fed Proc. 13:365.
18. Hess SM, Fitzhugh OG (1955). Absorption and excretion of certain triphenylmethane colours in rats and dogs. J. Pharmacol Exp Ther. 114:38–42.
19. Husain A, Sawaya W, Al-Omair A, Al-Zenki S, Al-Amiri H, Ahmed N et al. (2006). Estimates of dietary exposure of children to artificial food colours in Kuwait. Food Addit Contam. 23(3):245–51. Iga T, Awazu S, Nogami H (1971). Pharmacokinetic study of biliary excretion. II. Comparison of excretion behavior in triphenylmethane dyes. Chem Pharm Bull (Tokyo). 19(2):273–81.
20. Iga T, Awazu S, Hanano M, Nogami H (1971). Pharmacokinetic studies of biliary excretion. IV. The relationship between the biliary excretion behavior and the elimination from plasma of azo dyes and triphenylmethane dyes in rat. Chem Pharm Bull (Tokyo). 19(12):2609–16.
21. IRDC (1981a). FD&C Blue #1 – Long-term dietary toxicity/carcinogenicity study in mice. International Research and Development Corporation, Mattawan, MI, USA. Unpublished study report submitted by the International Association of Color Manufacturers (IACM) to WHO on 30 November 2016.
22. IRDC (1981b). FD&C Blue #1 – Long-term dietary toxicity/carcinogenicity study in rats. International Research and Development Corporation, Mattawan, MI, USA. Unpublished study report submitted by the International Association of Color Manufacturers (IACM) to WHO on 30 November 2016.
23. Ishidate M Jr, Sofuni T, Yoshikawa K, Hayashi M, Nohmi T, Sawada M et al. (1984). Primary mutagenicity screening of food additives currently used in Japan. Food Chem Toxicol. 22(8):623–36.
24. Kawachi T, Komatsu T, Kada T, Ishidate M, Sasaki M, Sugiyama T et al. (1980). Results of recent studies on the relevance of various short-term screening tests in Japan. In: Williams GM, Kroes R, Waaijers HW, van de Poll KW, editors. The predictive value of short-term screening tests in carcinogenicity evaluations. Amsterdam: Elsevier/North Holland Biomedical Press: 253–67.
25. Kreindler JJ, Slutsky J, Haddad ZH (1980). The effect of food colors and sodium benzoate on rat peritoneal mast cells. Ann Allergy. 44(2):76–81.
26. Kus E, Eroglu HE (2015). Genotoxic and cytotoxic effects of Sunset Yellow and Brilliant Blue, colorant food additives, on human blood lymphocytes. Pak J Pharm Sci. 28(1):227–30.
27. Lu FC, Lavallee A (1964). The acute toxicity of some synthetic colours used in drugs and foods. Can Pharm J. 97(12):30.
28. Lucová M, Hojerová J, Pažoureková S, Klimová Z (2013). Absorption of triphenylmethane dyes Brilliant Blue and Patent Blue through intact skin, shaven skin and lingual mucosa from daily life products. Food Chem Toxicol. 52:19–27.
29. Maloney KJ, Brand TA (2016). Absorption of FD&C Blue No. 1 from enteral feedings: A look back at a patient safety effort

with modern relevance. Pulm Crit Care Med. 1(2):52–7. doi:10.15761/PCCM.1000111.
30. Mannell WA, Grice HC, Allmark MG (1962). Chronic toxicity studies on food colours. V. Observations on the toxicity of Brilliant Blue FCF, Guinea Green B and Benzyl Violet 4B in rats. J Pharm Pharmacol. 14(1):378–84.
31. Pandir D (2016). DNA damage in human germ cell exposed to the some food additives in vitro. Cytotechnology. 68(4):725–33.
32. Phillips JC, Mendis D, Eason CT, Gangolli SD (1980). The metabolic disposition of ^{14}C-labelled green S and Brilliant Blue FCF in the rat, mouse and guinea-pig. Food Cosmet Toxicol. 18(1):7–13.
33. Rowland IR, Gaunt IF, Hardy J, Kiss IS, Butterworth KR (1975). Long-Term toxicity of Brilliant Blue FCF in mice. Research report no. 4. Unpublished report. Submitted to WHO by the International Association of Color Manufacturers (IACM).
34. Sasaki YF, Kawaguchi S, Kamaya A, Ohshita M, Kabasawa K, Iwama K et al. (2002). The comet assay with 8 mouse organs: results with 39 currently used food additives. Mutat Res. 519(1–2):103–19.
35. Tanaka T, Takahashi O, Inomata A, Ogata A, Nakae D (2012). Reproductive and neurobehavioral effects of brilliant blue FCF in mice. Birth Defects Res B Dev Reprod Toxicol. 95(6):395–409.
36. Wang J, Jackson DG, Dahl G (2013). The food dye FD&C Blue No. 1 is a selective inhibitor of the ATP release channel Panx1. J Gen Physiol. 141(5):649–56.
37. WHO (2003). Blue discoloration and death from FD&C Blue No. 1. WHO Drug Information. 17(4):239–40.
38. Waterman N, Lignac GO (1958). The influence of the feeding of a number of food colours on the occurrence of tumours in mice. Acta Physiol Pharmacol Neerl. 7(1):35–55.
39. Willheim R, Ivy AC (1953). A preliminary study concerning the possibility of dietary carcinogenesis. Gastroenterology. 23(1):1–19 [cited in Annex 1, reference *19*].

3.1.2 β-Carotene-rich extract from *Dunaliella salina*

Explanation

β-Carotene-rich extract from *Dunaliella salina* is a natural orange food colour. It is used as a colour in a wide range of food and beverages, including cider, malt beverages, water-based flavoured drinks, margarines, cheeses, cake fillings, custards, yogurts, processed nuts, precooked pastas and noodles and other products. Intended use levels of the product range from 20 mg/kg to 1200 mg/kg, depending on the food item or category.

Carotenes from natural sources (including carotenes from *D. salina*) were reviewed at the thirty-first, thirty-fifth and forty-first meetings of the Committee (Annex 1, references *77*, *88* and *107*). At the thirty-first meeting, the Committee concluded that the group ADI of 0–5 mg/kg bw established for the sum of the synthetic carotenoids β-carotene, β-apo-8′-carotenal and β-apo-8′-carotenoic acid methyl and ethyl esters by the eighteenth Committee was not applicable to natural carotenes as they did not comply with the specifications for β-carotene. At the thirty-fifth and forty-first meetings, the Committee considered the available data inadequate to establish an ADI for the dehydrated algal carotene preparations or for the vegetable oil extract of *D. salina*. At the fifty-seventh meeting, the group ADI for synthetic β-carotene was extended to include β-carotene from Blakeslea trispora (Annex 1, reference *154*).

The Committee was asked by the Forty-eighth Session of the CCFA (FAO/WHO, 2016) to evaluate carotenes from *D. salina*. New short-term animal studies as well as studies on genotoxicity and developmental toxicity were submitted.

A comprehensive literature search was conducted on carotenes from *D. salina*. The Committee also considered a limited number of publications on β-carotene from other sources that became available since the previous evaluation. In light of the information submitted, the Committee limited the assessment to a vegetable oil preparation of a β-carotene-rich d-limonene extract of *D. salina*, hereafter referred to as *D. salina* d-limonene extract.

Chemical and technical considerations

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

Carotenoids are naturally occurring pigments that are responsible for the bright colours of various fruits and vegetables, including citrus fruits, carrots and tomatoes. β-Carotene, a provitamin A, is the most common of these carotenoids, consisting of an unsaturated chain containing identical substituted ring structures at each end.

Biochemical aspects

β-Carotene is absorbed and detected in human serum and liver when consumed as an extract from *D. salina* or as synthetic β-carotene (Redlich et al., 1996). Peak levels of β-carotene in human serum occur between 24 and 48 hours after ingestion (Stahl, Schwarz & Sies, 1993). Absorption appears to be linear when doses up to 30 mg are ingested, but the degree of absorption decreases at higher concentrations (Woutersen et al., 1999). Absorption of β-carotene varies between 10% and 90% in humans and is dependent on various conditions, such as the food matrix and nutritional status of the individual (Wang et al., 1993; von Laar et al., 1996; Woutersen et al., 1999). In humans, the major storage sites for carotenoids are the liver and adipose tissue, and hepatic and adipose tissue levels tend to correlate with serum levels (Gaziano et al., 1995; Redlich et al., 1996). In

human serum, most of the β-carotene is present as the all-*trans* isomer, in spite of significant intake of the 9-*cis* isomer (Stahl, Schwarz & Sies, 1993; Rock, 1997; Woutersen et al., 1999).

In contrast to humans, mice, rats, hamsters and rabbits have very low levels of serum and tissue β-carotene due to the very high activity of intestinal β-carotene-15,15′-dioxygenase that efficiently converts β-carotene to retinal (During, Albaugh & Smith, 1998; During et al., 2001; Woutersen et al., 1999). On this basis, the Committee concluded that these species are not suitable models for the evaluation of β-carotene in humans.

The toxicokinetics of β-carotene in ferrets and preruminant calves have been shown to be similar to the absorption of β-carotene in humans. Ferrets that consumed 18 μmol/L β-carotene as a suspension in water for 16 days after a β-carotene elimination period of 10 days were shown to accumulate β-carotene in serum, liver and adrenal tissue (White et al., 1993). β-Carotene was also significantly increased in liver, spleen, lung and serum of preruminant calves fed a single oral dose of 20 mg. Serum levels were still elevated 264 hours post dosing (Poor et al., 1992).

Toxicological studies

At the present meeting, the Committee evaluated two new 90-day studies in rats, in vitro and in vivo genetic toxicity assays and a developmental toxicity study in rats conducted using a *D. salina* d-limonene extract. The Committee deemed these studies useful for evaluating the toxicity of the non-β-carotene portion of the extract.

In a 90-day study submitted to the Committee for this evaluation, rats were treated by gavage with *D. salina* d-limonene extract (containing 31% carotenes) at doses of 0, 318, 954 or 3180 mg/kg bw per day (calculated from doses of 0, 100, 300 and 1000 mg/kg bw per day carotenes using a correction factor of 3.18). Superficial erosion of the mucosa with infiltration of neutrophilic granulocytes and haemorrhages in the fundus region of the stomach were observed in one female and five males in the high-dose group (Leuschner, 2006a). The Committee concluded that the findings in the fundus were most likely due to a local effect of the high concentration of the test material given as a bolus, and identified the NOAEL to be 3180 mg/kg bw per day of *D. salina* d-limonene extract, the highest dose tested.

In another 90-day study, rats were fed diets containing 0%, 0.63%, 1.25%, 2.5% or 5% of an oil extract of carotenes from Dunaliella alga (species not specified). The Committee noted that although the test substance was not specified, based upon the reported percentage of β-carotene (31.4%) and the description of the material, this is likely a *D. salina* d-limonene extract. The average doses of the Dunaliella carotene extract were reported as 0, 352, 696,

1420 and 2750 mg/kg bw per day for males and 0, 370, 748, 1444 and 2879 mg/kg bw per day for females (Kuroiwa et al., 2006). Although the authors identified a NOAEL of 1.25% based on a 6% reduction of body weight gain in males at 2.5% and 5%, the Committee considered this not to be a toxicologically relevant effect. The Committee concluded that the NOAEL was 5% (2750 mg/kg bw per day) of Dunaliella extract, the highest concentration tested.

No long-term toxicity and carcinogenicity studies were available for *D. salina* d-limonene extract.

The *D. salina* d-limonene extract tested negative in genotoxicity assays, including the bacterial reverse mutation assay in five strains of *S. typhimurium*, the forward gene mutation assay in cultured mammalian cells ($TK^{+/-}$ L5178Y) with and without metabolic activation and an in vivo mouse bone marrow micronucleus test. No concerns for genotoxicity were identified (Leuschner, 2006b,c; Stien, 2006).

No reproductive toxicity studies were available for the *D. salina* d-limonene extract.

D. salina d-limonene extract (carotene content 31%) was administered to pregnant rats from gestation days 6 to 19 by oral gavage at doses of 0, 318, 954 or 3180 mg/kg bw per day of the extract (calculated from doses of 0, 100, 300 and 1000 mg/kg bw per day carotenes using a correction factor of 3.18). No maternal or developmental toxicity was observed (Leuschner, 2007).

Observations in humans

No studies were available on the *D. salina* d-limonene extract.

The Committee noted two independent trials of heavy smokers (at least 1 package/day for 36 years on average) who received β-carotene supplements. In the first study, participants received β-carotene (20 mg/day) supplementation, with or without α-tocopherol supplementation (Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study Group, 1994). In the second study, participants received β-carotene (30 mg/day) + retinol (25 000 International Units of vitamin A) (Omenn et al., 1996a,b). Both studies showed increased, rather than the hypothesized decreased, incidence of lung cancer. A subsequent systematic review of nine randomized clinical trials showed no increase in the incidence of lung cancer in heavy smokers at supplemental doses of β-carotene varying from 6 to 15 mg/day for about 5–7 years (Druesne-Pecollo et al., 2010).

Assessment of dietary exposure

The Committee considered dietary exposure to β-carotene from *D. salina* d-limonene extract assuming its uses as a food additive in the same food categories and at the same maximum use levels (β-carotene basis) as previously evaluated β-carotene additives. The Committee concluded that dietary exposure

to β-carotene would not change, as the extract will provide β-carotene at a level equivalent to that from other β-carotene food additives.

The Committee therefore reviewed dietary exposures to β-carotene reported in the literature. Estimates of dietary exposure from the following regions/countries were included in this review: Australia (Hodge et al., 2009), China (Chen et al., 2015; Wang et al., 2014), the Czech Republic (Stepaniak et al., 2016), "Europe" (Elmadfa, 2009; referenced in EFSA, 2012), France (Lassale et al., 2016), Italy (Sette et al., 2010), Japan (Yabuta et al., 2017), Republic of Korea (Kim et al., 2016), Poland (Kopeć et al., 2013; Stepaniak et al., 2016), Russian Federation (Stepaniak et al., 2016) and Spain (Beltrán-de-Miquel et al., 2015). As chemical analyses of β-carotene in food cannot distinguish β-carotene added to food from that occurring naturally, these dietary exposure estimates reflect total dietary exposure to β-carotene.

Overall, mean or median dietary exposures to β-carotene ranged from 1.4 to 11 mg/day in adults. For children, data from Europe showed a maximum mean exposure of 7.3 mg/day; globally, few data were available for children's dietary exposures. For high percentile consumers of foods containing β-carotene, dietary exposures were as high as 13.7 mg/day (adults in Europe).

The Committee concluded that a high daily dietary exposure to β-carotene of 15 mg (0.25 mg/kg bw for a 60 kg individual) is appropriate for use in safety assessment. Using this dietary exposure estimate and the assumptions that all the β-carotene in the diet comes from this extract and that the extract contains 30% β-carotene, 35% algal lipids (upper level of a range of 20–35%) and 0.3% d-limonene (maximum amount), dietary exposure to the other toxicologically relevant constituents of this extract would be 18 mg/day (0.3 mg/kg bw per day for a 60 kg individual) for algal lipids and 0.2 mg/day (0.003 mg/kg bw per day for a 60 kg individual) for d-limonene.

Evaluation

The Committee noted that the total dietary exposure to β-carotene is not expected to increase when *D. salina* d-limonene extract is used as a food colour.

The Committee has also considered the basis for the ADI established for the group of carotenoids by the Committee at the eighteenth meeting. The group ADI (0–5 mg/kg bw) was derived using a four-generation study in rats with a NOAEL for β-carotene of 50 mg/kg bw per day with application of a safety factor of 10 because of the natural occurrence of carotenoids in the human diet and the low toxicity observed in animal studies. This ADI applies to the use of β-carotene as a colouring agent and not to its use as a food supplement.

Data that have become available since the previous evaluation show large differences in absorption of β-carotene between rodent species and humans. Specific β-carotene-15,15′-dioxygenase activity with β-carotene as substrate

in the intestine of rodents is nearly 1 million-fold higher than that of humans. The Committee considered that rodents are inappropriate animal models for establishing an ADI for β-carotene because of the virtual absence of systemic absorption in rodents.

The Committee noted that the toxicity of the other components of the *D. salina* d-limonene extract can be evaluated using the results of rodent studies. The *D. salina* d-limonene extract used in the toxicological studies contained β-carotene at approximately 30%, algal lipids at 20–35% and diluent vegetable oil at 35–50%. The *D. salina* d-limonene extract did not show genotoxicity in the evaluated studies. Short-term toxicity studies in rats give a NOAEL equal to 3180 mg/kg bw per day, the highest dose tested. No effects were observed in a developmental toxicity study in rats. No long-term toxicity or reproductive studies have been conducted with the *D. salina* d-limonene extract. Correction of the dose used to derive the NOAEL for *D. salina* d-limonene extract of 3180 mg/kg bw per day for the percentage of the algal component (20–35%) gives an adjusted NOAEL of 636–1113 mg/kg bw per day for the algal lipid component of the test substance. The margin of exposure for the algal lipid component in the *D. salina* d-limonene extract is 2120–3710 using a dietary exposure of 18 mg/day (0.3 mg/kg bw per day). The Committee concluded that dietary exposure to the algal component of the extract does not pose a health concern.

The Committee concluded that there was no health concern for the use of β-carotene-rich extract from *D. salina* when used as a food colour and in accordance with the specifications established at this meeting. This conclusion was reached because total dietary exposure to β-carotene will not increase and there are no toxicity concerns for the non-carotene components of the extract. The Committee emphasized that this conclusion applies to the use of this extract as a food colour, not as a food supplement.

A toxicological and dietary exposure monograph was prepared.

A specifications monograph and a Chemical and Technical Assessment were prepared.

Recommendations

The Committee recommends that the group ADI for the sum of carotenoids, including β-carotene, β-apo-8′-carotenal and β-apo-8′-carotenoic acid methyl and ethyl esters, be re-evaluated in light of evidence that shows very low absorption of β-carotene in rodents and rabbits in contrast to humans.

References

1. Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study Group (1994). The effect of vitamin E and β-carotene on the incidence of lung cancer and other cancers in male smokers. N Engl J Med. 330(15):1029–35.

2. Beltrán-de-Miguel B, Estévez-Santiago R, Olmedilla-Alonso B (2015). Assessment of dietary vitamin A intake (retinol, α-carotene, β-carotene, β-cryptoxanthin) and its sources in the National Survey of Dietary Intake in Spain (2009–2010). Int J Food Sci Nutr. 66:706–12. doi:10.3109/09637486.2015.1077787.

3. Chen GD, Zhu YY, Cao Y, Liu J, Shi WQ, Liu ZM et al. (2015). Association of dietary consumption and serum levels of vitamin A and β-carotene with bone mineral density in Chinese adults. Bone. 79:110–5. doi:10.1016/j.bone.2015.05.028.

4. Druesne-Pecollo N, Latino-Martel P, Norat T, Barrandon E, Bertrais S, Galan P et al. (2010). Beta-carotene supplementation and cancer risk: A systematic review and metaanalysis of randomized controlled trials. Int J Cancer. 127(1):172–84.

5. During A, Albaugh G, Smith JC Jr (1998). Characterization of beta-carotene 15,15'-dioxygenase activity in TC7 clone of human intestinal cell line Caco-2. Biochem Biophys Res Commun. 249(2):467–74.

6. During A, Smith MK, Piper JB, Smith JC (2001). Beta-carotene 15,15'-dioxygenase activity in human tissues and cells: evidence of an iron dependency. J Nutr Biochem. 12(11):640–7.

7. EFSA (2012). Scientific Opinion on the reevaluation of mixed carotenes (E 160a (i)) and beta-Carotene (E 160a (ii)) as a food additive. EFSA J. 10(3):2593. doi:10.2903/j.efsa.2012.2593.

8. Elmadfa I (2009). European Nutrition and Health Report 2009. Forum Nutr. 62:I–XIII. doi:10.1159/000242366 [cited in EFSA, 2012].

9. FAO/WHO (2016). 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 Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission (REP16/FA).

10. Gaziano JM, Johnson EJ, Russell RM, Manson JE, Stampfer MJ, Ridker PM et al. (1995). Discrimination in absorption or transport of beta-carotene isomers after oral supplementation with either all-trans- or 9-cis-beta-carotene. Am J Clin Nutr. 61(6):1248–52.

11. Hodge AM, Simpson JA, Fridman M, Rowley K, English DR, Giles GG et al. (2009). Evaluation of an FFQ for assessment of antioxidant intake using plasma biomarkers in an ethnically diverse population. Public Health Nutr. 12:2437–47. doi:10.1017/S1368980009005539.

12. Kim DE, Cho SH, Park HM, Chang YK (2016). Relationship between bone mineral density and dietary intake of β-carotene, vitamin C, zinc and vegetables in postmenopausal Korean women: a cross-sectional study. J Int Med Res. 44:1103–14. doi:10.1177/0300060516662402.

13. Kopeć A, Cieślik E, Leszczyńska T, Filipiak-Florkiewicz A, Wielgos B, Piątkowska E et al. (2013). Assessment of polyphenols, beta-carotene, and vitamin C intake with daily diets by primary school children. Ecol Food Nutr. 52:21–33. doi:10.1080/03670244.2012.705766.

14. Kuroiwa Y, Nishikawa A, Imazawa T, Kitamura Y, Kanki K, Ishii Y et al. (2006). A subchronic toxicity study of dunaliella carotene in F344 rats. Food Chem Toxicol. 44(1):138–45.

15. Lassale C, Castetbon K, Laporte F, Deschamps V, Vernay M, Camilleri GM et al. (2016). Correlations between fruit, vegetables, fish, vitamins, and fatty acids estimated by web-based nonconsecutive dietary records and respective biomarkers of nutritional status. J Acad Nutr Diet. 116:427–38. doi:10.1016/j.jand.2015.09.017.

16. Leuschner PJ (2006a). Repeated-dose 90-day oral toxicity study of C-SAT050080 in rats. Unpublished study conducted by LPT Laboratory of Pharmacology and Toxicology KG, Hamburg, Germany, 10 August 2006. Submitted to WHO by BASF.

17. Leuschner PJ (2006b). Mutagenicity study of C-SAT050080 in the mouse lymphoma forward mutation assay (in vitro). Unpublished study conducted by LPT Laboratory of Pharmacology and Toxicology KG, Hamburg, Germany, 11 April 2006. Submitted to WHO by BASF.

18. Leuschner PJ (2006c). Mutagenicity study of C-SAT050080 in the *Salmonella typhimurium* reverse mutation assay (in vitro). Unpublished study conducted by LPT Laboratory of Pharmacology and Toxicology KG, Hamburg, Germany, 1 March 2006. Submitted to WHO by BASF.

19. Leuschner PJ (2007). Prenatal developmental toxicity study of C-SAT050080 in rats by oral administration. Unpublished study conducted by LPT Laboratory of Pharmacology and Toxicology KG, Hamburg, Germany. Submitted to WHO by BASF.

20. Omenn GS, Goodman GE, Thornquist MD, Balmes J, Cullen MR, Glass A et al. (1996a). Risk factors for lung cancer and for intervention effects in CARET, the Beta-Carotene and Retinol Efficacy Trial. J Natl Cancer Inst. 88(21):1550–59.

21. Omenn GS, Goodman G, Thornquist M, Barnhart S, Balmes J, Cherniack M et al. (1996b). Chemoprevention of lung cancer: The Beta-Carotene and Retinol Efficacy Trial (CARET) in high-risk smokers and asbestos-exposed workers. IARC Sci Publ. (136):67–85.

22. Poor CL, Bierer TL, Merchen NR, Fahey GC Jr, Murphy MR, Erdman JW Jr (1992). Evaluation of the preruminant calf as a model for the study of human carotenoid metabolism. J Nutr. 122(2):262–8.

23. Redlich CA, Grauer JN, Van Bennekum AM, Clever SL, Ponn RB, Blaner WS (1996). Characterization of carotenoid, vitamin A, and alpha-tocopheral levels in human lung tissue and pulmonary macrophages. Am J Respir Crit Care Med. 154(5):1436–43.

24. Rock CL (1997). Carotenoids: biology and treatment. Pharmacol Ther. 75(3):185–97.

25. Sette S, Le Donne C, Piccinelli R, Arcella D, Turrini A, Leclercq C et al. (2010). The third Italian National Food Consumption Survey, INRAN-SCAI 2005e06 e Part 1: Nutrient intakes in Italy. Nutr Metab Cardiovasc Dis. 21:922–32. doi:10.1016/j.numecd.2010.03.001.

26. Stahl W, Schwarz W, Sies H (1993). Human serum concentrations of all-trans beta- and alpha-carotene but not 9-cis beta-carotene increase upon ingestion of a natural isomer mixture obtained from Dunaliella salina (Betatene). J Nutr. 123(5):847–51.

27. Stien J (2006). Mutagenicity study of C-SAT050080 in the *Salmonella typhimurium* reverse mutation assay (in vitro). Unpublished study conducted by LPT Laboratory of Pharmacology and Toxicology KG, Hamburg, Germany. Submitted to WHO by BASF.

28. Stepaniak U, Micek A, Grosso G, Stefler D, Topor-Madry R, Kubinova R et al. (2016). Antioxidant vitamin intake and mortality in three Central and Eastern European urban populations: the HAPIEE study. Eur J Nutr. 55:547–60. doi:10.1007/s00394–015–0871–8.

29. von Laar J, Stahl W, Bolsen K, Goerz G, Sies H (1996). β-Carotene serum levels in patients with erythropoietic protoporphyria on treatment with the synthetic all-trans isomer or a natural isomeric mixture of β-carotene. J Photochem Photobiol B. 33(2):157–62.

30. Wang XD, Russell RM, Marini RP, Tang G, Dolnikowski GG, Fox JG et al. (1993). Intestinal perfusion of beta-carotene in the ferret raises retinoic acid level in portal blood. Biochim Biophys Acta. 1167(2):159–64.

31. Wang L, Li B, Pan MX, Mo XF, Chen YM, Zhang CX (2014). Specific carotenoid intake is inversely associated with the risk of breast cancer among Chinese women. Br J Nutr. 111(9):1686–95. doi:10.1017/S000711451300411X.

32. White WS, Peck KM, Ulman EA, Erdman JW Jr (1993). Evaluation of the bioavailability of natural and synthetic forms of beta-carotenes in a ferret model. Ann N Y Acad Sci. 691(1):229–31.

33. Woutersen RA, Wolterbeek AP, Appel MJ, van den Berg H, Goldbohm RA, Feron VJ (1999). Safety evaluation of synthetic beta-carotene. Crit Rev Toxicol. 29(6):515–42.

34. Yabuta S, Urata M, Yap R, Kun W, Masaki M, Shidoji Y (2016). Common SNP rs6564851 in the BC01 Gene affects the circulating levels of β-carotene and the daily intake of carotenoids in healthy Japanese women. PLoS One. 11:e0168857. doi:10.1371/journal.pone.0168857.

3.1.3 Fast Green FCF

Explanation

Fast Green FCF (CAS No. 2353-45-9; INS No. 143) is a dye with a synthetic triphenylmethane base structure permitted as a food colour in Japan, the USA and other regions. It is used for colouring breakfast cereals, cakes and cupcakes, drink mixers and frozen treats.

The Committee previously evaluated Fast Green FCF at its thirteenth, twenty-fifth, twenty-ninth and thirtieth meetings (Annex 1, references *19*, *56*, *70* and *73*). At the thirteenth meeting, the Committee established an ADI of 0–12.5 mg/kg bw based on a long-term feeding study in rats. At the twenty-fifth meeting, the ADI of 0–12.5 mg/kg bw was made temporary pending the results of adequate long-term feeding studies and a multigeneration reproduction/developmental study. At the twenty-ninth meeting, two long-term toxicity and carcinogenicity studies and a three-generation reproductive study were available for review. It was noted that a mouse oral carcinogenicity study was negative, but that an increased incidence of urothelial hyperplasia and/or neoplasia of the bladder was observed at the highest dose in the rat study. The biological significance of observed differences in benign and malignant tumours at other sites was considered questionable since, apart from the bladder, complete histopathological examinations were not performed on the low- and intermediate-dose animals. The temporary ADI was extended to permit complete histopathological examination of all groups of rats and biometric examination of the data. At the thirtieth meeting, the Committee reviewed histopathological data from the rat oral carcinogenicity study and concluded that Fast Green FCF was noncarcinogenic in rats and established an ADI of 0–25 mg/kg bw, based on a long-term study of toxicity in rats.

At the present meeting, the Committee re-evaluated this food colour at the request of the Forty-eighth Session of the CCFA (FAO/WHO, 2016).

A toxicological dossier was submitted that included new studies on genotoxicity and neurological effects, and a search of the literature was

conducted that yielded one additional study relevant for the present evaluation. This Committee also considered studies evaluated at previous meetings of the Committee (before 1986).

Chemical and technical considerations

Fast Green FCF consists mainly of disodium 3-[*N*-ethyl-*N*-[4-[[4-[*N*-ethyl-*N*-(3-sulfobenzyl)amino]phenyl](4-hydroxy-2-sulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]ammoniomethyl]-benzenesulfonate and its isomers, together with subsidiary colouring matters, as well as sodium chloride and/or sodium sulfate as the principal uncoloured components. It is manufactured by condensing 2-formylhydroxybenzenesulfonic acid with a mixture of 3-[(*N*-ethyl-*N*-phenylamino)methyl]benzenesulfonic acid and its 2- and 4-isomers to form the leuco base precursor. Oxidation of the leuco base precursor with either chromium- or manganese-containing compounds produces the dye, which is isolated as the disodium salt. The dye contains not less than 85% total colouring matters. Impurities include unreacted starting material and reaction by-products (approximately 2%), subsidiary colouring matters (≤6%), residual leuco base precursor (≤5%), unsulfonated primary aromatic amines (≤0.01% calculated as aniline), lead (≤2 mg/kg), chromium (≤50 mg/kg) and manganese (≤100 mg/kg).

Biochemical aspects

Absorption of orally administered Fast Green FCF was shown to be less than 5%; almost all the administered colour was excreted unchanged in the faeces of the rats (Hess & Fitzhugh, 1953, 1954, 1955).

In an in vitro study, in which the *Xenopus* oocyte expression system was used for pharmacological investigations on purinergic P2 receptors that interact with the membrane channel protein pannexin 1 (Panx1) in inflammasome signalling, Fast Green FCF was shown to be a selective inhibitor of pannexin 1 (Panx1) channels, with an IC_{50} of 0.27 μmol/L, and did not significantly inhibit the P2X7R receptor (Wang, Jackson & Dahl, 2013). The Committee was aware that Panx1 activation/inhibition is one of several signalling pathways involved in various physiological processes at the cellular level (e.g. immune function) and that there are many exogenous and endogenous modulators of these pathways. Interactions of substances with P2 receptors and Panx1 are an active area of research, particularly in development of drug treatments for diverse chronic diseases. The Committee noted that a similar pattern of channel inhibition was observed with Brilliant Blue FCF, and further research may clarify if the inhibition of Panx1 observed in an in vitro system has any relevance for the safety assessment for substances in food.

Toxicological studies

Fast Green FCF has low oral acute toxicity in rats (Lu & Lavallee, 1964) and dogs (Radomski & Deichman, 1956).

A short-term study of toxicity revealed no compound-related effects in dogs fed Fast Green FCF at 0%, 1.0% or 2.0% of the diet (equal to 0, 269 and 695 mg/kg bw per day, respectively) for 2 years (Hansen et al., 1966).

Two previously reviewed long-term studies of oral toxicity showed no compound-related effects in mice and rats. The NOAEL was 2% Fast Green FCF in the diet (equivalent to 3000 mg/kg bw per day) in mice and 5.0% (equivalent to 2500 mg/kg bw per day) in rats (Hansen et al., 1966).

No treatment-related increase in tumour incidence was found in a mouse carcinogenicity study (Hogan & Knezevich, 1981). At the twenty-ninth meeting, the Committee concluded that the NOAEL was 5% Fast Green FCF in the diet, the highest dose tested. The present Committee noted that the mean body weights of females in the 5% dose group were consistently lower than those of controls after the commencement of the study (−10% compared with relevant controls at termination of the study). The Committee considered this decrease in body weights to be a treatment-related adverse effect and concluded that the NOAEL was 1.5% Fast Green FCF (equal to 3392 mg/kg bw per day), based on the lower body weights observed at 5% (equal to 11 805 mg/kg bw per day) in females.

A carcinogenicity study in rats reported an increased incidence of urothelial hyperplasia and/or neoplasia of the bladder (Knezevich & Hogan, 1981). However, a peer review of the histopathological data showed that Fast Green FCF is noncarcinogenic in this species (Dua, Chowdury & Moch, 1982; O'Donnell, 1982; USFDA, 1982a,b). The previous Committee agreed with this conclusion at its thirtieth meeting and concluded that the NOAEL in this dietary study was 5% Fast Green FCF (equal to 3184 mg/kg bw per day), the highest dose tested (Annex 1, reference *73*). The present Committee concurred with this conclusion.

Whereas 10 of the 18 available genotoxicity tests were negative, four in vitro and four in vivo studies yielded positive results. Given that all of the studies with positive test outcomes had several limitations in experimental design and reporting, whereas an in vivo mouse bone marrow micronucleus assay (Hayashi et al., 1988) and an in vivo mouse tissue comet assay (Sasaki et al., 2002) were clearly negative, the Committee concluded that there is no concern with respect to genotoxicity of Fast Green FCF.

No reproductive toxicity was reported at doses up to 1000 mg/kg bw per day over three generations of rats (Smith, 1973).

No developmental toxicity studies were available. However, information on the developmental toxicity of the structurally related substance Brilliant Blue

FCF, which differs from Fast Green FCF by a single hydroxyl group, was available. No developmental toxicity was reported in rats treated with Brilliant Blue FCF at doses up to 2000 mg/kg bw per day or in rabbits at up to 200 mg/kg bw per day (BioDynamics Inc. 1972a,b). Based on these findings, the Committee concluded that there is no concern for developmental toxicity for Fast Green FCF.

Observations in humans

No data were available.

Assessment of dietary exposure

Estimates of dietary exposure to Fast Green FCF published by the Republic of Korea (Ha et al.. 2013) and the USFDA (Doell et al., 2016) were available. Because the estimates were based on only a few findings in a limited number of food groups, the Committee conducted a conservative assessment using the FAO/WHO Chronic individual food consumption database – Summary statistics (CIFOCOss) database and Codex maximum levels.

Dietary exposure to Fast Green FCF was estimated to be 12 mg/kg bw per day for adolescents, the age group with the highest exposure, at the 95th percentile. This estimate was much higher than those of both the USFDA (0.09 mg/kg bw per day for children at the 90th percentile) and the Republic of Korea (0.003 mg/kg bw per day for the whole population at the 95th percentile). The Committee concluded that these differences were due to the use of Codex maximum levels, in contrast to the estimates from the USFDA and the Republic of Korea, which used mean analysed levels for all foods.

The Committee concluded that the conservative estimate of 12 mg/kg bw per day, prepared using CIFOCOss data, should be considered in the safety assessment for Fast Green FCF.

Evaluation

The Committee at previous meetings concluded that Fast Green FCF is not carcinogenic. The evidence newly available at this meeting indicates that there is no concern with respect to genotoxicity of Fast Green FCF. The ADI of 0–25 mg/kg bw established previously by the Committee was based on a long-term rat dietary study in which a NOAEL of 5% Fast Green FCF (equivalent to 2500 mg/kg bw per day), the highest concentration tested, was identified (Hansen et al., 1966).

The Committee concluded that the new data that had become available since the previous evaluation gave no reason to revise the ADI and confirmed the ADI of 0–25 mg/kg bw. The Committee noted that the conservative dietary exposure estimate for Fast Green FCF of 12 mg/kg bw per day (95th percentile for adolescents) was below the upper bound of the ADI. The Committee concluded

that dietary exposures to Fast Green FCF for adolescents and all other age groups do not present a health concern.

A toxicological and dietary exposure monograph was prepared.

At the present meeting, the existing specifications for Fast Green FCF were revised, and a maximum limit for manganese was added. HPLC methods were added to determine subsidiary colouring matters and organic compounds other than colouring matters. The assay method was changed to visible spectrophotometry, and spectrophotometric data were provided for the colour dissolved in water or aqueous ammonium acetate.

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

References

1. BioDynamics Inc. (1972a). FD&C Blue #1 Segment II. Rabbit teratology study. Project no. 71R-721C. Unpublished report submitted to WHO by BioDynamics Inc., East Millstone, NJ, USA.
2. BioDynamics Inc. (1972b). FD&C Blue #1 Segment II. Rat teratology study. Project no. 71R-719C. Unpublished report submitted to WHO by BioDynamics Inc., East Millstone, NJ, USA.
3. Doell DL, Folmer DE, Lee HS, Butts KM, Carberry SE (2016). Exposure estimate for FD&C colour additives for the US population. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 33(5):782–97. doi:org/10.1080/19440049.2016.1179536.
4. Ha MS, Ha SD, Choi SH, Bae DH (2013). Exposure assessment of synthetic colours approved in Korea. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 30(4):643–53. doi:o rg/10.1080/19440049.2013.768358.
5. Dua PN, Chowdury KA, Moch RW (1982). Pathology report on FD&C Green No. 3. Unpublished pathology report, project no. PR-75, CAP no. 8C0065. Submitted to WHO by the USFDA.
6. FAO/WHO (2016). 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 Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission (REP16/FA).
7. Hansen WH, Long EL, Davis KJ, Nelson AA, Fitzhugh OG (1966). Chronic toxicity of three food colourings: guinea green B, light green SF yellowish, and fast green FCF in rats, dogs and mice, Food Cosmet Toxicol. 4:389–410.
8. Hayashi M, Kishi M, Sofuni T, Ishidate M (1988). Micronucleus tests in mice on 39 food additives and eight miscellaneous chemicals. Food Chem Toxicol. 26:487–500.
9. Hess SM, Fitzhugh OG (1953). Metabolism of coal-tar dyes. I. Triphenylmethane dyes. Fed Proc. 12:330–1.
10. Hess SM, Fitzhugh OG (1954). Metabolism of coal-tar dyes. II. Bile studies. Fed Proc. 13:365.
11. Hess SM, Fitzhugh OG (1955). Absorption and excretion of certain triphenylmethane colours in rats and dogs. J Pharmacol Exp Ther. 114:38–42.

12. Hogan GK, Knezevich AL (1981). A long-term oral carcinogenicity study of FD&C Green No. 3 in mice. Unpublished report No. 77–1781 from BioDynamics Inc., East Millstone, NJ, USA. Submitted to WHO by Certified Color Manufacturers' Association.

13. Knezevich AL, Hogan GK (1981). A long-term oral toxicity/carcinogenicity study of FD&C Green No. 3 in rats. Unpublished report No. 77–1780 from BioDynamics Inc., East Millstone, NJ, USA. Submitted to WHO by Certified Color Manufacturers' Association.

14. Lu FC, Lavallee A (1964). The acute toxicity of some synthetic colours used in drugs and food. Can Pharm J. 97:30.

15. O'Donnell MW (1982). Memorandum. Long-term oral toxicity/carcinogenicity studies of FD&C Green No. 3 in rats. Unpublished report, project no. 77–1780. Submitted to WHO by the USFDA.

16. Radomski JL, Deichman WB (1956). Cathartic action and metabolism of certain coal tar food dyes. J Pharmacol Exp Ther. 118:322–7.

17. Sasaki YF, Kawaguchi S, Kamaya A, Ohshita M, Kabasawa K, Iwama K et al. (2002). The comet assay with 8 mouse organs: results with 39 currently used food additives. Mutat Res. 519:103–19.

18. Smith JM (1973). A three-generation reproduction study of FD&C Green No. 3 in rats. Unpublished report No. 71R-736 from BioDynamics Inc., East Millstone, NJ, USA. Submitted to WHO by Certified Colour Manufacturers' Association.

19. USFDA (1982a). Memorandum of conferences Feb. 4 and Aug. 30, 1982 of the Cancer Assessment Committee. Submitted to WHO by the USFDA [cited in Annex 1, reference *74*].

20. USFDA (1982b). Federal Register, 47, 52140–52145 [cited in Annex 1, reference *74*].

21. Wang J, Jackson DG, Dahl G (2013). The food dye FD&C Blue No. 1 is a selective inhibitor of the ATP release channel Panx1. J Gen Physiol. 141(5):649–56.

3.1.4 Gum ghatti

Explanation

Gum ghatti (CAS No. 9000-26-6), also known as Indian gum, ghatti gum or gum ghati, is the dried gummy exudate from wounds in the bark of *Anogeissus latifolia* Wallich (family Combretaceae), a large tree native to India and Sri Lanka (Al-Assaf, Phillips & Amar, 2009). Gum ghatti is used as a thickener and stabilizer. It is permitted as a food additive in Japan and the USA.

Gum ghatti was previously evaluated at the twenty-sixth and twenty-ninth JECFA meetings (Annex 1, references *59* and *70*). Heavy metal specifications were revised at the fifty-seventh JECFA meeting (Annex 1, reference *154*). No ADI could be established at the twenty-sixth or twenty-ninth meetings because of insufficient data, but the Committee did not make specific recommendations for further studies; no monographs were prepared.

At the present meeting, the Committee evaluated gum ghatti at the request of the Forty-eighth Session of the CCFA (FAO/WHO, 2016). A toxicological dossier was submitted. Two new 90-day rat studies as well as genotoxicity studies

have become available since the previous evaluations. To address any data gaps for gum ghatti, the safety data on other polysaccharide-based gums were considered based on their similar general structure, chemical and functional properties, technical uses, lack of absorption as intact substances and their metabolism in the lower gastrointestinal tract.

A comprehensive literature search up to April 2017 was performed in PubMed and TOXLINE. Although the search resulted in five additional papers, these did not add further relevant data to those submitted to the Committee for this meeting.

Chemical and technical considerations

Unprocessed gum ghatti occurs as both amorphous "tears" of various sizes and as broken irregular pieces. It is light to dark brown in colour, has little or no odour and is available commercially in the form of brown tears or grey to reddish-grey powder. The product in commerce is manufactured by collecting the dried translucent exudate as tears, partially dissolving these in water and filtering. The final product is sterilized and dried to a gummy, lump form or spray-dried to a powder form.

Gum ghatti consists mainly of calcium (or occasionally magnesium) salts of high molecular weight and water-soluble complex polysaccharides. The hydrolysis of the polysaccharide yields L-arabinose, D-galactose, D-glucuronic acid and D-mannose, and small amounts of D-xylose and L-rhamnose. The reported average molar ratio of the various units is L-arabinose:D-galactose:D-glucuronic acid:D-mannose:D-xylose:L-rhamnose = 40:25:20:7:1:1 (Sakai et al., 2013). Gum ghatti also contains protein-bound arabinogalactan units, tannins and moisture. The weight average molecular weight of gum ghatti is in the order of several hundred kDa (Kang et al., 2015).

Biochemical aspects

Absorption, distribution, metabolism and excretion data on gum ghatti were not available. However, similar to other gums and dietary fibres, gum ghatti is unlikely to be significantly digested or absorbed in the stomach or small intestine. Based on its chemical composition, gum ghatti is expected to be enzymatically degraded and fermented by the microflora in the large intestine to hydrogen gas, carbon dioxide and short-chain fatty acids, which can be absorbed and metabolized (Ali, Ziada & Blunden, 2009).

Toxicological studies

In an acute toxicity study in male rats (Newell & Maxwell, 1972), no deaths were reported at 10 000 mg/kg bw, the highest dose tested.

Two new 90-day studies of the toxicity of gum ghatti (purity 85%) have been performed in rats.

In the first study (Davis & Lea, 2011), rats were fed a basal diet (AIN-93M) containing 0%, 0.5%, 1.5% or 5% gum ghatti (equal to 0, 337, 1018 and 3044 mg/kg bw for males and 0, 396, 1149 and 3308 mg/kg bw per day for females, respectively). Although haematological and clinical chemistry effects were observed, they were not dose related, not found in both sexes and/or not correlated with any histopathological findings.

Increased caecal weights were observed in the male and female rats at 5% gum ghatti. In addition, in 6 out of 10 high-dose males, minimal to mild mucosal hyperplasia and/or minimal to mild crypt elongation were observed in the caecum, whereas no lesions were found in the caecum of female rats. In 2 out of 10 high-dose females, ulcerative colitis was observed in the colon; no significant lesions were observed in the colon of male rats.

In order to evaluate the relevance of the ulcerative colitis, the possible role of the AlN-93M diet and the possibility that intrinsically susceptible littermates had been randomly assigned to the same group, a second study tested two different basal diets (AIN-93M and NIH-07) containing 0% or 5% gum ghatti (equal to 0 and 3671 mg/kg bw per day for rats fed the AIN-93M diet and 0 and 3825 mg/kg bw per day for rats fed the NIH-07 diet) (Davis, 2012). This study deviated from Organisation for Economic Co-operation and Development (OECD) Test Guideline 408, as only one dose was tested in only one sex (female) and the histopathological examination did not include the full range of recommended organs, because the aim of this second study was to follow up on the observations reported in female rats at the highest dose in the first study.

Increased empty caecal weights were observed in animals exposed to gum ghatti in both diets. Full caecal weights (absolute and relative) were also increased in rats exposed to gum ghatti in the AIN-93M diet but not the NIH-07 diet. Focal lymphoid hyperplasia of the colon was observed in all study groups, but there was no association with the dietary exposure to gum ghatti, and the authors concluded that these findings were incidental and not treatment related. A pathology working group subsequently concluded that the ulcerative colitis observed in the colon of the female rats was a sporadic event not associated with the dietary exposure to gum ghatti and that the caecal changes in male rats could not be confirmed as caecal crypt hyperplasia/crypt elongation (Maronpot et al., 2013).

The Committee noted that the effects on caecal weights observed in both sexes at 5% gum ghatti have also been reported in other toxicity studies of poorly digestible polysaccharides and gum products (Tulung, Rémésy & Demigné, 1987; Wyatt et al., 1988; Levrat et al., 1991; Doi et al., 2006; Ali, Ziada & Blunden, 2009; Hagiwara et al., 2010). The increase in caecal weight is considered to be the result

of microbial fermentation of undigested and unabsorbed gum in the lower large intestine (Newberne, Conner & Estes, 1988). The Committee considered this increase in caecal weight at 5% dietary gum ghatti to be an adaptive, rather than adverse, response. Based on the results of the new 90-day studies (Davis & Lea, 2011; Davis, 2012), the Committee identified a NOAEL of 3044 mg/kg bw per day (equal to 2590 mg/kg bw per day, corrected for purity), the highest dose tested.

No long-term studies of the toxicity or carcinogenicity of gum ghatti were available.

In vitro and in vivo genotoxicity studies of gum ghatti have recently been conducted. These, together with earlier in vitro and in vivo studies, showed no evidence for a genotoxic potential of gum ghatti. The Committee concluded that there were no genotoxicity concerns for gum ghatti.

No reproductive toxicity studies were available for gum ghatti.

Studies on developmental toxicity of gum ghatti administered by oral gavage were performed in mice, rats, hamsters and rabbits (Food and Drug Research Laboratories, Inc., 1972a). In mice and hamsters dosed at 0, 17, 80, 370 or 1700 mg/kg bw per day, there were no treatment-related adverse effects on the dams. There were also no treatment-related adverse effects on the numbers of implantations, resorptions or live and dead fetuses or on the frequency of external, soft tissue or skeletal abnormalities.

In the rat, there were four, zero, one, one and five maternal deaths at 0, 17, 80, 370 and 1700 mg/kg bw per day, respectively. Severe diarrhoea and urinary incontinence with anorexia were observed in the 2–3 days prior to death. Petechial haemorrhage was observed in the mucosa of the small intestine of the dams that died. There were no treatment-related adverse embryo-fetal effects at any dose, including in those rats that survived at the highest dose tested.

In the rabbit study, there were 15 animals per dose group. There were 3, 0, 3, 5 and 10 maternal deaths in the 0, 7, 33, 150 and 700 mg/kg bw per day dose groups, respectively. As with rats, severe diarrhoea and urinary incontinence with anorexia were observed in the 2–3 days prior to death. In addition, all animals aborted prior to death. Petechial haemorrhage was observed in the mucosa of the small intestine of the does that died. There were no treatment-related adverse embryo-fetal effects at any dose, including in the two pregnant rabbits that survived at the highest dose tested.

These developmental toxicity studies were performed prior to OECD guidelines or good laboratory practice (GLP) standards and do not comply with several modern standards/guidelines: the purity of the substance was not stated; the treatment period covered the major phase of organogenesis but did not extend to the end of gestation; and the rationale for dose selection in all four studies was not presented. In addition, none of the study reports presented the clinical

observations, feed consumption, gravid uteri weights or statistical analyses of the results. The Committee also noted that there were maternal deaths at high doses in mice, rats and, in particular, rabbits in developmental toxicity studies on other gums conducted by the same laboratory at about the same time, in which the test substance was also administered by oral gavage. The Committee considered that this may have been due to the difficulty of administering high concentrations of viscous substances by gavage. They further noted that no treatment-related adverse maternal or developmental effects were reported in surviving high-dose animals in studies on gum ghatti and other gums. Despite the deficiencies in the study methods and reporting and the occurrence of maternal deaths, there were no effects on embryo-fetal growth or development at doses up to 1700 mg/kg bw per day.

In view of the gaps in the database for gum ghatti (i.e. the absence of any long-term toxicity or carcinogenicity studies, the limitations of the developmental toxicity studies and the lack of any reproductive studies), the Committee considered data on structurally related gums. The gum most closely related to gum ghatti is gum arabic (also known as gum acacia); the two gums have similar monosaccharide profiles with respect to L-arabinose, L-rhamnose, D-galactose and D-glucuronic acid (Pitthard & Finch, 2001; Akiyama, Yamazaki & Tanamoto, 2011).

Developmental toxicity studies on other gums in mice, rats, hamsters and rabbits (Food and Drug Research Laboratories Inc., 1972b) were conducted by the same laboratory that conducted the developmental toxicity studies on gum ghatti. As such, they may have had similar limitations. A more recent combined fertility and developmental toxicity study of gum arabic in rats (Collins et al., 1987) was previously evaluated by the Committee, which considered that this study did not give cause for concern about the safety of gum arabic (Annex 1, reference *89*). EFSA (EFSA, 2017) also described more recent fertility studies in rats (Morseth & Ihara, 1989; Huynh et al., 2000) and considered that these studies did not give cause for concern about the safety of gum arabic. Based on the combined fertility and developmental toxicity study in rats (Collins et al., 1987), an overall NOAEL of 10 647 mg/kg bw per day (the highest dose tested) was identified for reproductive, developmental and parental effects. The Committee noted that reproductive and developmental toxicity studies on other gums (carob bean gum [FAS 16], cassia gum [FAS 62], gellan gum [FAS 28], guar gum [FAS 8], karaya gum [FAS 24]), tara gum [FAS 21]), tragacanth [FAS 20]), xanthan gum [FAS 21]) also previously evaluated by the Committee (Annex 1, references *57*, *197*, *95*, *39*, *84*, *74*, *72* and *74*) did not raise any health concerns for reproductive or developmental effects.

Overall, the Committee concluded that there were no health concerns for gum ghatti regarding reproductive or developmental effects.

Previously evaluated carcinogenicity studies of gum arabic in mice and rats conducted by the United States National Toxicology Program (National Toxicology Program, 1982) found no indications of any treatment-related increases in tumour incidence at dietary gum arabic concentrations of 2.5% and 5.0% (equivalent to 1250 and 2500 mg/kg bw per day for rats and 3750 and 7500 mg/kg bw per day for mice). The Committee noted that other previously evaluated chronic toxicity/carcinogenicity studies in mice and rats (carob bean gum, gellan gum, guar gum, tara gum, xanthan gum) also raised no health concerns regarding carcinogenic potential.

Observations in humans

No observations of gum ghatti in humans were available. However, three human studies on gum arabic found that daily ingestion by adults of up to 30 g (equivalent to 500 mg/kg bw per day for a 60 kg individual) over 18–21 days was well tolerated (Ross et al., 1983; Sharma, 1985; Cherbut et al., 2003). Furthermore, Ross et al. (1983) found that gum arabic could not be detected in the stool, indicating complete fermentation in the colon.

Assessment of dietary exposure

The Committee received one assessment of dietary exposure to gum ghatti from the sponsor and prepared estimates of dietary exposure based on model diets and potential use scenarios using food consumption data from the European Union and the USA.

The sponsor's submission noted that gum ghatti is used in a number of countries. The only use levels reported were from the USA. The Committee was unable to find information on the typical use levels in other countries. The submission to the Committee contained two reports outlining use levels for gum ghatti in a number of GSFA food categories. The Committee prepared estimates of dietary exposure based on these levels.

One report contains use levels for foods in GSFA categories 1.1.4 "Flavoured fluid milk drinks" and categories 14.1 and 14.2 (various beverage categories). The maximum use level for milk beverages was 150 mg/L; for non-alcoholic beverages, 100 mg/L; and for alcoholic beverages, 300 mg/L. Using food consumption data from the USA, the Committee completed a scenario assessment of dietary exposure by assuming 250 g/day consumption of milk beverages (95th percentile); 900 g/day of non-alcoholic beverages (95th percentile); and 750 g/day of alcoholic beverages (95th percentile). The estimated dietary exposure to gum ghatti would be 350 mg/day or 6 mg/kg bw per day for a 60 kg individual. The use of these maximizing assumptions in the preparation of the estimate from

these three broad food groups results in a highly conservative estimate of chronic dietary exposure to gum ghatti.

The second report contains a more extensive list of foods potentially containing gum ghatti. The Committee concluded that only consumption of noodles containing gum ghatti at a use level of 6000 mg/kg diet would result in a dietary exposure different from that in the scenario discussed above. The dietary exposure to gum ghatti from consumption of 60 g of prepared noodles containing the maximum level would be approximately 360 mg/day (6 mg/kg bw per day for a 60 kg individual), doubling the previous scenario estimate (12 mg/kg bw per day).

The Committee also used the EFSA Food Additive Intake Model (Version 1.0) with the use levels from the sponsor's report to estimate dietary exposure to gum ghatti. The estimated exposure for adults was 6 mg/kg bw per day.

The Committee considered that a dietary exposure of 12 mg/kg bw per day was suitable for use in a safety assessment of gum ghatti.

Evaluation

Because limited toxicological data on gum ghatti were available, ADIs were not established at previous meetings (Annex 1, references *59* and *70*). The present Committee evaluated two new 90-day studies in rats that did not show adverse effects at doses up to 3044 mg/kg bw per day, the highest dose tested (equal to 2590 mg/kg bw per day when corrected for purity). The Committee took into account the lack of systemic exposure to gum ghatti because of its high molecular weight and polysaccharide structure, its lack of toxicity in short-term studies, the lack of concern for genotoxicity and the absence of treatment-related adverse effects in studies of gum arabic and other polysaccharide gums with a similar profile.

The Committee concluded that gum ghatti is unlikely to be a health concern and established an ADI "not specified" for gum ghatti that complies with the specifications.

Therefore, the Committee concluded that the estimated dietary exposure to gum ghatti of 12 mg/kg bw per day does not represent a health concern.

A consolidated monograph was prepared.

The specifications were revised based on submitted information and available literature. An HPLC method for the identification of the gum constituents was added to replace the thin-layer chromatography method. One identity method, using a mercury-containing reagent, was removed. L- Rhamnose was added as one of the constituents of gum ghatti, based on current literature reports.

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

References

1. Akiyama T, Yamazaki T, Tanamoto K (2011). [Analysis of thickening polysaccharides by the improved diethyldithioacetal derivatization method]. Shokuhin Eiseigaku Zasshi. 52(1):40–6 (in Japanese).
2. Al-Assaf S, Phillips GO, Amar V (2009). Gum ghatti. In: Phillips GO, Williams PA. Handbook of hydrocolloids, second edition: A volume in Woodhead Publishing Series in Food Science, Technology and Nutrition. Oxford: Woodhead Publishing Limited; 477–94.
3. Ali BH, Ziada A, Blunden G (2009). Biological effects of gum arabic: A review of some recent research. Food Chem Toxicol. 47(1):1–8.
4. Cherbut C, Michel C, Raison V, Kravtchenko T, Severine M (2003). Acacia gum is a bifidogenic dietary fibre with high digestive tolerance in healthy humans. Microb Ecol Health Dis. 15:43–50.
5. Collins TF, Welsh JJ, Black TN, Graham SL, Brown LH (1987). Study of the teratogenic potential of gum arabic. Food Chem Toxicol. 25(11):815–21 [cited in Annex 1, reference *89*].
6. Davis JP, Lea IA. (2011). Subchronic oral toxicity study with gum ghatti in Sprague-Dawley rats. Unpublished report by ILC, Inc, Study no.: C185–002.
7. Davis JP (2012). Repeat subchronic oral toxicity study with gum ghatti in female Sprague-Dawley rats. Unpublished report by ILC, Inc, Study no.: C185–011.
8. Doi Y, Ichihara T, Hagiwara A, Imai N, Tamano S, Orikoshi H et al. (2006). A ninety-day oral toxicity study of a new type of processed gum arabic, from Acacia tree (*Acacia senegal*) exudates, in F344 rats. Food Chem Toxicol. 44(4):560–6.
9. EFSA (2017). Re-evaluation of acacia gum (E414) as a food additive. EFSA J. 15(4):4741–92.
10. FAO/WHO (2016). 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 Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission (REP 16/FA).
11. Food and Drug Research Laboratories, Inc. (1972a). Teratologic evaluation of FDA 71–13 (Gum Ghatti). Unpublished report to the US Food and Drug Administration, Report no. FDABF-GRAS-066. Submitted to WHO by the International Association of Color Manufacturers (IACM).
12. Food and Drug Research Laboratories, Inc. (1972b). Teratologic evaluation of FDA 71–15 (gum arabic) gum in mice, rats, hamsters and rabbits. Report no. FDABF-GRAS-063 Aug 1972.
13. Hagiwara A, Imai N, Doi Y, Sano M, Tamano S, Omoto T et al. (2010). Ninety-day oral toxicity study of rhamsan gum, a natural food thickener produced from Sphingomonas ATCC 31961, in Crl:CD(SD)IGS rats. J Toxicol Sci. 35(4):493–501.
14. Huynh PN, Hikim AP, Wang C, Stefonovic K, Lue YH, Leung A et al. (2000). Long-term effects of triptolide on spermatogenesis, epididymal sperm function, and fertility in male rats. J Androl. 21:689–99.
15. Kang J, Guo Q, Wang Q, Phillips GO, Cui SW (2015). New studies on gum ghatti (*Anogeissus latifolia*) part 5. The conformational properties of gum ghatti. Food Hydrocoll. 43:25–30.
16. Levrat MA, Behr SR, Rémésy C, Demigné C (1991). Effects of soybean fiber on cecal digestion in rats previously adapted to a fiber-free diet. J Nutr. 121(5):672–8.
17. Maronpot RR, Davis, J, Moser G, Giri DK, Hayashi SM (2013). Evaluation of 90-day oral rat toxicity studies on the food additive, gum ghatti. Food Chem Toxicol. 51:215–24.

18. Morseth SL, Ihara T (1989). Reproduction study in rats with manidipine hydrochloride (CV-4093 (2 HCl)). Yakuri To Chiryo. 17:145–62.

19. National Toxicology Program (1982). Carcinogenesis bioassay of gum arabic (CAS No. 9000-01-5) in F344/N rats and B6C3F1/N mice (feed study). Technical Report Series No. 227. NIH Publication No. 82–1783. Washington (DC): U.S. Department of Health & Human Services [cited in Annex 1, reference *89*].

20. Newberne PM, Conner MW, Estes P (1988). The influence of food additives and related materials on lower bowel structure and function. Toxicol Pathol. 16(2):184–97.

21. Newell GW, Maxwell WA (1972). Study of the mutagenic effects of gum ghatti (FDA No. 71–13). Unpublished report no. FDABF-GRAS-088 to the US Food and Drug Administration, SRI International, Menlo Park, CA, USA. Submitted to WHO by the International Association of Color Manufacturers (IACM).

22. Pitthard V, Finch P (2001). GC-MS analysis of monosaccharide mixtures as their diethyldithioacetal derivatives: applications to plant gums used in art work. Chromatographia. 53(Suppl 1):S317–21.

23. Ross AH, Eastwood MA, Brydon WG, Anderson JR, Anderson DM (1983). A study of dietary gum Arabic in humans. Am J Clin Nutr. 37:368–75.

24. Sakai E, Katayama T, Ogasawara T, Mizuno M (2013). Identificationi of *Anogeissus latifolia* Wallich and analysis of refined gum ghatti. J Nat Med. 67:276–80.

25. Sharma RD (1985). Hypocholesterolemic effects of gum acacia in men. Nutr Res. 5:1321–6.

26. Tulung B, Rémésy C, Demigné C (1987). Specific effect of guar gum or gum arabic on adaptation of cecal digestion to high fiber diets in the rat. J Nutr. 117(9):1556–61.

27. Wyatt GM, Horn M, Gee JM, Johnson IT (1988). Intestinal microflora and gastrointestinal adaptation in the rat in response to non-digestible dietary polysaccharides. Br J Nutr. 60(2):197–207.

3.1.5 Jagua (Genipin–Glycine) Blue

Explanation

Jagua (Genipin–Glycine) Blue (CAS No. 1314879-21-4) is the product of the reaction between stoichiometric equivalents of genipin extracted from the unripe *Genipa americana* Linne (Rubiaceae) fruit and glycine, resulting in a blue-coloured genipin–glycine polymer and dimers. This report refers to the blue-coloured genipin–glycine polymer and dimer content of Jagua (Genipin–Glycine) Blue as the "blue polymer" content. *G. americana* fruit has traditionally been used for the preparation of juices, jellies, marmalades and liquors (Ramos-de-la-Peña et al., 2015).

Jagua (Genipin–Glycine) Blue is permitted for use as a food colour in Colombia.

Jagua (Genipin–Glycine) Blue has not been evaluated previously by the Committee. It was on the agenda at the request of the Forty-eighth Session of the CCFA (FAO/WHO, 2016).

The sponsor provided a dossier containing chemical, technical, dietary exposure and toxicological data, including unpublished in vitro studies, genotoxicity studies and in vivo toxicological studies in rats and dogs.

A comprehensive literature search on *G. americana* and the related colour Gardenia Blue was conducted. One long-term toxicity study on Gardenia Blue was identified and added to the toxicological data submitted to the Committee for this meeting.

The name was changed from "Jagua extract" to "Jagua (Genipin–Glycine) Blue" because the name "Jagua extract" was not adequately descriptive.

Chemical and technical considerations

G. americana L. is a small to medium-sized tree (UNCTAD, 2005) that belongs to the Rubiaceae family and is native to central and tropical South America (Djerassi, Gray & Kincl, 1960; Ueda, Iwahashi & Tokuda, 1990). The plant yields edible berries referred to as jagua fruit, chipara, guayatil, maluco, caruto or huito (Ramos-de-la-Peña et al., 2015) in Spanish and as genipap in English.

The unripe jagua fruit contains high levels of a cyclopentan-[C]-pyran skeleton class of compound, called iridoids (Dinda, Debnath & Harigaya, 2007a,b). Genipin is a unique iridoid in its ability to crosslink with primary amines present in amino acids and proteins, in the presence of oxygen, to produce high molecular weight water-soluble blue pigments (Fujikawa et al., 1987; Touyama et al., 1994a,b; Paik et al., 2001; Park et al., 2002; Cho et al., 2006; Lee, Lee & Jeong, 2009).

The deep blue/black colour of Jagua (Genipin–Glycine) Blue is obtained by treating peeled and ground pulp of unripe fruits of *G. americana* L. with water. The resulting juice is filtered and treated with a stoichiometric amount of glycine based on the concentration of genipin in the water extract; it is heated at 70 °C for 2 hours, until the blue colour is completely formed. The product is centrifuged, concentrated and/or dried. Unreacted genipin is considered an impurity of Jagua (Genipin–Glycine) Blue. The liquid product is obtained by concentrating the Jagua (Genipin–Glycine) Blue up to 20–50°Bx and formulating with food-grade glycerine or other permitted food additives. Alternatively, a powder is obtained, after concentrating the Jagua (Genipin–Glycine) Blue to 20°Bx, mixing with a food-grade carrier, then spray-drying and sieving.

The Jagua (Genipin–Glycine) Blue product in commerce contains a blue polymer (20–40%) and three blue dimers (approximately 1.5%) as colouring matters. The remaining components of the product are carbohydrates (>55%), protein (approximately 7%) and water (approximately 5%). The blue polymer composed of repeating dimers has the molecular formula $(C_{27}H_{25}O_8N_2)_n$ and an average molecular weight of 6000 Da. The molecular formulae of the three identified dimers are $C_{28}H_{28}O_8N_2$ (CAS No. 1313734-13-2), $C_{27}H_{25}O_8N_2$ (CAS

No. 104359-67-3) and $C_{27}H_{24}O_8N_2$ (CAS No. 1313734-14-3). The blue polymer and the three dimers have been identified by nuclear magnetic resonance spectroscopy (^1H, ^{13}C), infrared spectroscopy, mass spectroscopy and HPLC. The Jagua (Genipin–Glycine) Blue is stable and has no decomposition products under normal storage conditions.

Biochemical aspects

The molecular weight and chemical properties of the "blue polymer" of Jagua (Genipin–Glycine) Blue suggest that the polymer is unlikely to be absorbed intact from the gastrointestinal tract. A size distribution analysis showed that less than 1.5% of the mixture contained dimers with molecular weights of around 500 Da, which could be absorbed. An in vitro study using a Caco-2 cell intestinal barrier model showed that "blue polymer" has poor passive penetration, but there is some evidence to suggest that a small proportion of Jagua (Genipin–Glycine) Blue, possibly the smallest coloured molecular species (such as genipin–glycine dimers of molecular weight approximately 500 Da, or other coloured low molecular weight components), was actively transported (Gilbert, 2015). No "blue polymer" was detectable in the plasma of dogs in an oral gavage repeated-dose study (tested up to 338 mg/kg bw per day, limit of quantification 1 mg/mL) on day 1 or 91 following dosing with Jagua (Genipin–Glycine) Blue (Mancari, 2016). No investigations into biotransformation of the "blue polymer" were undertaken.

Toxicological studies

Results from an oral gavage acute toxicity test in the rat showed no adverse effects at the highest tested dose of 660 mg/kg bw (Allingham, 2014).

Results from oral gavage 90-day repeated-dose toxicity studies in rats and dogs showed no adverse effects at 330 mg/kg bw per day or 338 mg/kg bw per day of "blue polymer", respectively, the highest doses tested (Allingham et al., 2014; Mancari, 2016). The dog study deviated from the relevant OECD test guideline, but the Committee considered these deviations to be minor and to not affect the validity of the study. The Committee noted that in dogs, the urine was coloured green with an intensity that appeared to be in proportion to the administered dose, and there was an increase in measured serum bilirubin values attributed to the interference of the test article with the analytical method, suggesting that some of the "blue polymer" had been absorbed from the gastrointestinal tract. Green-coloured urine was not observed in rats. In all 90-day animal studies, all treated animals had faeces that were coloured blue, which is consistent with poor absorption of the high molecular weight component of the "blue polymer" from the gastrointestinal tract.

There were no long-term toxicity or carcinogenicity studies available on Jagua (Genipin–Glycine) Blue. To address the data gap, one non-GLP carcinogenicity study in rats on a structurally related genipin-based blue polymer from *Gardenia jasminoides* (Gardenia Blue) was considered. The Gardenia Blue used in the study was formed from a mixture of genipin and a protease digest of soy proteins, resulting in different amino acids attached to genipin. The Committee noted that the purity of blue polymer in the Gardenia Blue was not described. At concentrations up to 5% in the diet (equal to 2173 mg/kg bw in the males and 2533 mg/kg bw in the females), there were no treatment-related adverse effects or changes in tumour incidence (Imazawa et al., 2000).

There was no evidence of genotoxicity of Jagua (Genipin–Glycine) Blue in vitro, with bacterial reverse mutation assays and a mouse lymphoma assay, or in vivo, with a mouse micronucleus assay. The Committee concluded that there was no concern with regard to genotoxicity.

No reproductive or developmental toxicity studies on Jagua (Genipin–Glycine) Blue were available; there were also no available reproductive or developmental toxicity studies on Gardenia Blue.

Observations in humans

No relevant human studies were available.

Assessment of dietary exposure

Estimates of dietary exposure to Jagua (Genipin–Glycine) Blue prepared by the sponsor based on dietary data for the United States population, estimated use levels and use frequencies were available to the Committee. In addition, a conservative assessment using the CIFOCOss database and maximum use levels provided by the sponsor was performed by the Committee. The 95th percentile estimates of dietary exposure for Jagua (Genipin–Glycine) Blue on a "blue polymer" basis calculated by the Committee were 11 mg/kg bw per day for children and 5 mg/kg bw per day for adolescents. These estimates were much higher than those calculated by the sponsor. The difference between the sponsor's estimates and the Committee's estimates was due to the use by the sponsor of lower use levels and use frequencies.

The Committee concluded that the conservative estimate of 11 mg/kg bw per day for children and 5 mg/kg bw per day (for adolescents), prepared using the CIFOCOss model, should be considered in the safety assessment for Jagua (Genipin–Glycine) Blue on a "blue polymer" basis.

Evaluation

The Committee noted that in 90-day toxicity studies with Jagua (Genipin–Glycine) Blue in the dog and rat, no treatment-related adverse effects were found at the highest doses tested; in addition, genotoxicity tests were negative, and no treatment-related adverse effects were observed in a carcinogenicity study with the structurally related food colour, Gardenia Blue. Based on the coloration of the urine in the dogs and the increase in serum bilirubin test values, which was attributed to interference of the test article with the analytical method, the Committee concluded that some component of the Jagua (Genipin–Glycine) Blue is absorbed and excreted, most likely the dimers or other coloured low molecular weight component. (The dimers make up less than 1.5% of Jagua (Genipin–Glycine) Blue). However, the Committee noted that the highest doses tested in both 90-day studies were only 330 and 338 mg/kg bw per day (expressed on a "blue polymer" basis) in rats and dogs, respectively. The Committee was concerned that the possible effects of the low molecular weight species that could be absorbed would not have been adequately investigated.

A comparison of the dietary exposure estimate (11 mg/kg bw per day) with the NOAEL from the 90-day studies of oral toxicity in rats and dogs (approximately 330 mg/kg bw per day) gives a margin of exposure of approximately 30.

Because of the limited biochemical and toxicological database and the low margin of exposure, the Committee was unable to complete the evaluation for Jagua (Genipin–Glycine) Blue.

A toxicological and dietary exposure monograph was prepared.

A new tentative specifications monograph and a Chemical and Technical Assessment were prepared.

The Committee raised concern regarding the potential toxicity of low molecular weight fraction of the total colouring matter in Jagua (Genipin–Glycine) Blue. The Committee recommends additional biochemical and toxicological studies (e.g. absorption, distribution, metabolism and excretion studies, long-term toxicity, carcinogenicity, reproductive and developmental toxicity studies), including on the use of higher doses of the "blue polymer", including the dimers, in order to complete an evaluation of the safety of Jagua (Genipin–Glycine) Blue.

To support the above, additional information is required on:

- Characterization of the low molecular weight components of the "blue polymer";
- A validated method for the determination of the dimers; and
- Data on concentrations of dimers from five batches of the commercial product.

References

1. Allingham P (2014). Acute oral toxicity (fixed dose procedure) limit test with Jagua extract. Report no. 136354. Unpublished report by BSL Bioservice, Planegg, Germany. Submitted to WHO by Ecoflora SAS, Columbia.
2. Allingham P, Weise K, Weber K, Janku S (2014). 90-Day repeated-dose oral toxicity study in Wistar rats with jagua extract including a 28-day recovery period. Report no. 132041. Unpublished report by BSL Bioservice, Planegg, Germany. Submitted to WHO by Ecoflora SAS, Columbia.
3. Cho YJ, Kim SY, Kim J, Choe EK, Kim SI, Shin HJ (2006). One-step enzymatic synthesis of blue pigments from geniposide for fabric dyeing. Biotechnol Bioprocess Eng. 11(3):230–4.
4. Dinda B, Debnath S, Harigaya Y (2007a). Naturally occurring iridoids. A review, part 1. Chem Pharm Bull (Tokyo). 55(2):159–222.
5. Dinda B, Debnath S, Harigaya Y (2007b). Naturally occurring secoiridoids and bioactivity of naturally occurring iridoids and secoiridoids. A review, part 2. Chem Pharm Bull (Tokyo). 55(5):689–728.
6. Djerassi C, Gray JD, Kincl FA (1960). Naturally occurring oxygen heterocyclics. IX. 1 Isolation and characterization of Genipin 2. J Org Chem. 25(12):2174–7.
7. FAO/WHO (2016). 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 Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission (REP16/FA).
8. Fujikawa S, Fukui Y, Koga K, Kumada J (1987). Brilliant skyblue pigment formation from gardenia fruits. J Ferment Technol. 65(4):419–24.
9. Gilbert J (2015). Caco-2 permeability testing of Jagua extract. Study no. CYP1291-R1. Unpublished report performed by Cryprotex US, LLC, Watertown, MA, USA. Prepared for Ricerca Biosciences, Concord, OH, USA. Submitted to WHO by Ecoflora SAS, Columbia.
10. Imazawa T, Nishikawa A, Furukawa F, Kasahara K, Ikeda T, Takahashi M et al. (2000). Lack of carcinogenicity of gardenia blue colour given chronically in the diet to F344 rats. Food Chem Toxicol. 38(4):313–8.
11. Lee JH, Lee DU, Jeong CS (2009). *Gardenia jasminoides* Ellis ethanol extract and its constituents reduce the risks of gastritis and reverse gastric lesions in rats. Food Chem Toxicol. 47(6):1127–31.
12. Mancari F (2016). Jagua extract: 90-Day oral toxicity study in the dog. Study no. 2015–0175. Unpublished report performed by Accelera S.R.L., Nerviano, Milan, Italy. Sponsored by AnaPath GmbH, on behalf of Ecoflora. Submitted to WHO by Ecoflora SAS, Columbia.
13. Paik Y, Lee C, Cho M, Hahn T (2001). Physical stability of the blue pigments formed from geniposide of gardenia fruits: effects of pH, temperature, and light. J Agric Food Chem. 49(1):430–2.
14. Park JE, Lee JY, Kim HG, Hahn TR, Paik YS (2002). Isolation and characterization of water-soluble intermediates of blue pigments transformed from geniposide of Gardenia jasminoides. J Agric Food Chem. 50(22):6511–4.
15. Ramos-de-la-Peña AM, Montañez JC, de la Luz Reyes-Vega M, Hendrickx ME, Contreras-Esquivel JC (2015). Recovery of genipin from genipap fruit by high pressure processing. Food Sci Technol-LEB. 63(2):1347–50.

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

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

18. Ueda S, Iwahashi Y, Tokuda H (1991). Production of anti-tumor-promoting iridoid glucosides in *Genipa americana* and its cell cultures. J Nat Prod. 54(6):1677–80.

19. UNCTAD (2005). World Investment Report 2005: Transnational corporations and the internationalization of R&D. Geneva: United Nations Conference on Trade and Development.

3.1.6 Metatartaric acid

Explanation

Metatartaric acid (CAS No. 56959-20-7/39469-81-3; INS No. 353), a polymer of L(+)-tartaric acid, is used as a food additive in winemaking in the following countries and regions: Argentina, Australia, Brazil, Canada, Chile, the European Union, New Zealand, Norway, Paraguay, Russian Federation, South Africa, Turkey and Uruguay.

Metatartaric acid, which was not previously evaluated by the Committee, was evaluated at the request of the CCFA at its Forty-eighth Session (FAO/WHO, 2016). It is proposed for use in winemaking at a level of good manufacturing practice. The data that were submitted in response to the call for data related to its use as a food additive in winemaking only.

The safety of L(+)-tartaric acid and DL-tartaric acid and their sodium and potassium salts was evaluated at the seventeenth and twenty-first meetings of the Committee (Annex 1, references *32* and *44*). At its seventeenth meeting, the Committee established a group ADI of 0–30 mg/kg bw for L(+)-tartaric acid and its sodium, potassium and potassium–sodium salts, expressed as L(+)-tartaric acid. At its twenty-first meeting, the Committee reaffirmed the ADI for the L(+)-tartrate monosodium salt and the existing specifications for L(+)-tartaric acid, but did not establish an ADI for DL-tartrate monosodium salt.

L(+)-Tartaric acid, the naturally occurring form of tartaric acid, occurs in many fruits and wines. Tartrate crystals (potassium bitartrate and calcium tartrate) develop naturally in wine and are the major cause of sediment in bottled wines. In order to prevent sedimentation, metatartaric acid has been used in wine since 1955 (OIV, 2012; Guise et al., 2014).

At the present meeting, the Committee reviewed a short-term toxicity study and a genotoxicity study of metatartaric acid. A literature search was conducted, but no studies relevant for the safety assessment of metatartaric acid were identified. However, for L(+)-tartaric acid, three other toxicity studies were

identified and evaluated. Studies on L(+)-tartaric acid from 1977 onward had not been previously reviewed by the Committee.

Chemical and technical considerations

Metatartaric acid is typically manufactured using L(+)-tartaric acid from natural sources. It is formed by the intermolecular esterification between the carboxylic group of one L-tartaric acid unit and the secondary alcohol group of another molecule of L-tartaric acid, which may be followed by further intermolecular and intramolecular esterification reactions (Sprenger et al., 2015). The primary components of metatartaric acid are the L-tartaric acid monomer, ditartrate monoester and diester, and polyester chains of varying degrees of polymerization. The average molecular weight range has been determined in commercial products to be 2.2–8.9 kDa, with a polydispersity index up to 50. Metatartaric acid is used as a stabilizer and sequestrant in wine to prevent growth and precipitation of potassium bitartrate and calcium tartrate crystals (Marchal & Jeandet, 2009). Stability studies in wine indicated that it undergoes hydrolysis to tartaric acid over time, but the rate of hydrolysis is dependent on pH and storage temperature (Ribéreau-Gayon et al., 2006; Morello, 2012).

Metatartaric acid is produced by heating L-tartaric acid from grapes at 150–170 °C under atmospheric or reduced pressure for less than 1 hour (Ribéreau-Gayon et al., 2006). This process produces a colourless liquid, which is cooled, dried and ground into an off-white powder. Variations in production temperature, pressure and time allow manufacturers to alter the degree of esterification in the final product.

Biochemical aspects

Metatartaric acid

Metatartaric acid, a polydisperse polymer of tartaric acid units linked together by ester bonds, is anticipated to undergo rapid enzyme-mediated hydrolysis to L(+)-tartaric acid once exposed to carboxylesterases in the gastrointestinal tract.

L(+)-Tartaric acid

The disposition of L(+)-tartaric acid following ingestion appears to differ markedly between most of the animal species investigated (rats, rabbits, dogs and pigs) and humans. In rats, rabbits, dogs and pigs, most of the ingested tartrate is absorbed and excreted unchanged (50–100%) in the urine (Underhill et al., 1931; Gry & Larsen, 1978). The extent of absorption and urinary excretion of unchanged tartrate in guinea-pigs (13–27%) is similar to that observed in humans (12%) (Underhill et al., 1931; Chadwick et al., 1978).

In rats, 15–22% of ingested tartrate is exhaled as carbon dioxide. Microbial fermentation was confirmed following intracaecal administration, when 66% of the administered dose (18.8 mg/kg bw) was exhaled as radiolabelled carbon dioxide, while less than 2% of the administered dose was absorbed and excreted in urine (Chasseaud, Down & Kirkpatrick, 1977; Chadwick et al., 1978). In rats, the concentration–time curve for radiolabelled L(+)-tartrate suggested a short half-life in plasma of around 3 hours (Down et al., 1977).

Apart from its excretion in urine, there is evidence of extensive microbial fermentation of L(+)-tartaric acid to carbon dioxide in humans: very little unchanged tartrate (<5%) has been detected in faeces. Although the concentration of radiolabelled carbon dioxide exhaled by humans 1 hour after intravenous dosing was small (18%), suggesting metabolism by tissue enzymes, up to 46% of the label was exhaled 4 hours after oral dosing (Chadwick et al., 1978).

Toxicological studies

Metatartaric acid

No acute toxicity studies were available.

Rats exposed to metatartaric acid in their drinking-water at concentrations up to 3.0% for 18 weeks had markedly reduced body weight due to a dose-related reduction in feed and water intake, owing to the poor palatability of metatartaric acid in water at all concentrations tested (Ingram et al., 1982). As a result, the Committee considered this study to be unsuitable for a safety assessment of metatartaric acid.

Metatartaric acid was not genotoxic in a reverse mutation assay.

No long-term toxicity and carcinogenicity, reproductive toxicity or developmental toxicity studies were available.

L(+)-Tartaric acid

The LD_{50} of sodium tartrate in mice was reported to be 4360 mg/kg bw; for disodium tartrate in male rabbits, it was greater than 3680 mg/kg bw (Locke et al., 1942).

The Committee noted that no new long-term toxicity studies had become available since the previous evaluation of L(+)-tartaric acid. However, the previously unpublished toxicity study that supports the ADI for tartaric acid had since been published. In that study, no treatment-related adverse effects were observed in rats with diets containing monosodium L(+)-tartrate at concentrations of 0, 25 600, 42 240, 60 160 or 76 800 mg/kg bw (reported to be equal to L(+)-tartaric acid doses of 0, 710, 1220, 1840 and 2460 mg/kg bw per day for males and 0, 930, 1600, 2360 and 3200 mg/kg bw per day for females, respectively) (Hunter et al., 1977). The Committee noted that the conversion reported in the publication used the molecular weight for disodium tartrate

rather than monosodium tartrate to calculate the doses of L(+)-tartaric acid. Using monosodium tartrate, the Committee calculated the doses to be 0, 770, 1400, 1900 and 2680 mg/kg bw per day for males and 0, 1030, 1780, 2630 and 3550 mg/kg bw per day for females, respectively. The Committee concluded that the NOAEL for L(+)-tartaric acid in the study was 2680 mg/kg bw per day, the highest tested dose.

In two in vitro assays including reverse mutation (*S. typhimurium* strains TA92, TA1535, TA100, TA1537, TA94 and TA98) and chromosomal aberration (Chinese hamster fibroblast cell line), L(+)-tartaric acid showed no genotoxic potential at concentrations up to 1 mg/mL. However, although sodium L(+)-tartrate was negative in the reverse mutation assay, it was positive in a chromosomal aberration test at high concentrations of up to 15 mg/mL (Ishidate et al., 1984). The Committee noted that no testing of potential cytotoxicity was performed and that gaps had been counted in the chromosomal aberration test. The Committee concluded that these factors call into question the reliability of this study. In addition, the related compound, L(+)-tartaric acid, at 1 mg/mL was shown to be negative in the same assay. Sodium L(+)-tartrate was also negative using single intraperitoneal doses up to 3600 mg/kg bw in an in vivo micronucleus test in mice (Hayashi et al., 1988).

Assessment of dietary exposure

The sponsor requested the use of metatartaric acid as a food additive in wine at a maximum use level of 100 mg/L. The Committee conducted international dietary exposure assessments for metatartaric acid in wine using the GEMS/Food cluster diets database. The dietary exposure estimate for metatartaric acid ranged from 0.0004 (G14) to 0.2 mg/kg bw per day (G7) (per capita), assuming a 60 kg body weight and 100 mg/L of metatartaric acid as the maximum use level. The Committee also prepared international estimates of dietary exposure to metatartaric acid using wine (food category 14.2.3.1 "Still grape wine" and food category 14.2.3.3 "Fortified grape wine, grape liquor wine and sweet grape wine") consumption levels from the CIFOCOss database and 100 mg/L of metatartaric acid as the maximum use level. The estimates of mean dietary exposure to metatartaric acid for adult consumers of wine ranged up to 0.3 mg/kg bw per day, and the highest 95th percentile dietary exposures in adult consumers of wine reached 0.8 mg/kg bw per day. The Committee prepared dietary estimates to metatartaric acid in wine using consumption data from the 1995 Australian National Nutrition Survey, the 1997 New Zealand National Nutrition Survey and the USA National Health and Nutrition Examination Surveys, with the maximum use level of 100 mg/L. These estimates were 1.3, 1.3 and 0.3 mg/kg bw per day for the 95th percentile exposures for adult consumers of wine, respectively.

The Committee assumed that metatartaric acid hydrolyses to an approximately equivalent concentration of tartaric acid. The Committee noted that the dietary exposure to metatartaric acid for the highest 95th percentile adult consumers of wine (1.3 mg/kg bw per day, expressed as L(+)-tartaric acid) is appropriate for use in this safety assessment.

Evaluation

As metatartaric acid undergoes enzymatic hydrolysis to tartaric acid prior to systemic absorption, the biochemical and toxicological data on tartaric acid considered at previous meetings are relevant to the safety assessment of the metatartaric acid. Additional information to support the safety assessment of metatartaric acid includes the absence of any effects in a bacterial reverse mutation test. The present Committee evaluated a series of studies that had become available since L(+)-tartaric acid was last evaluated. The body of evidence suggests no change to the group ADI previously established for L(+)-tartaric acid and its sodium, potassium and potassium–sodium salts, expressed as L(+)-tartaric acid.

The Committee concluded that metatartaric acid (when used in winemaking) should be included in the group ADI of 0–30 mg/kg bw for L(+)-tartaric acid and its sodium, potassium and potassium–sodium salts, expressed as L(+)-tartaric acid.

The Committee noted that the dietary exposure estimate for metatartaric acid for adult consumers of wine was 4% of the upper bound of the ADI and concluded that dietary exposure to metatartaric acid in wine at the maximum use level of 100 mg/L does not present a health concern.

A toxicological and dietary exposure monograph was prepared.

Tentative specifications and a Chemical and Technical Assessment were prepared.

The Committee received limited analytical data on metatartaric acid. In order to remove the tentative designation from the specifications, the following information on the products of commerce is requested:

- Characterization of the products (optical rotation, content of free tartaric acid, degree of esterification and molecular weight distribution) and the corresponding analytical methods;
- Infrared spectrum (in a suitable medium); and
- Analytical results including the above parameters from a minimum of five batches of products currently available in commerce, along with quality control data.

The Committee requests that this information be submitted by December 2018.

References

1. Chasseaud LF, Down WH, Kirkpatrick D (1977). Absorption and biotransformation of L (+)-tartaric acid in rats. Experientia. 33:998–9.
2. Chadwick VS, Vince A, Killingley M, Wrong OM (1978). The metabolism of tartrate in man and the rat. Clin Sci Mol Med. 54(3):273–81.
3. Down WH, Sacharin RM, Chasseaud LF, Kirkpatrick D, Franklin ER (1977). Renal and bone uptake of tartaric acid in rats: Comparison of L (+) and DL-Forms. Toxicology. 8(3):333–46.
4. FAO/WHO (2016). 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 Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission (REP 16/FA).
5. Gry J, Larsen JC (1978). Metabolism of L (+)-and D (-)-tartaric acids in different animal species. Arch Toxicol Suppl. 1:351–3.
6. Guise R, Filipe-Ribeiro L, Nascimento D, Bessa O, Nunes FM, Cosme F (2014). Comparison between different types of carboxymethylcellulose and other oenological additives used for white wine tartaric acid stabilization. Food Chem. 156:250–7.
7. Hayashi M, Kishi M, Sofuni T, Ishidate M Jr (1988). Micronucleus tests in mice on 39 food additives and eight miscellaneous chemicals. Food Chem Toxicol. 26(6):487–500.
8. Hunter B, Batham P, Heywood R, Street AE, Prentice DE (1977). Monosodium L (+) tartrate toxicity in two year dietary feeding to rats. Toxicology. 8(2):263–74.
9. Ingram AJ, Butterworth KR, Gaunt IF, Gangolli SD (1982). Short-term toxicity study of metatartaric acid in rats. Food Chem Toxicol. 20:253–7.
10. Ishidate M Jr, Sofuni T, Yoshikawa K, Hayashi M, Nohmi T, Sawada M et al. (1984). Primary mutagenicity screening of food additives currently used in Japan. Food Chem Toxicol. 22(8):623–36.
11. Locke A, Locke RB, Schlesinger H, Carr H (1942). The comparative toxicity and cathartic efficiency of disodium tartrate and fumarate, and magnesium fumarate, for the mouse and rabbit. J Am Pharm Assoc. 31(1):12–4.
12. Morello A (2012). Influence of pH and temperature on metatartaric acid efficiency in white wine tartaric stabilization [thesis]. Lisbon:University of Lisbon.
13. Marchal R, Jeandet P (2009). Use of enological additives for colloid and tartrate salt stabilization in white wines and for improvement of sparkling wine foaming properties. In: Moreno-Arribas MV, Polo MC, editors. Wine chemistry and biochemistry. New York: Springer:127–58.
14. OIV (2012). International Code of Oenological Practices. 2012 Issue. Paris: Organisation International de la Vigne et du Vin.
15. Ribéreau-Gayon P, Glories Y, Maujean A, Dubourdieu D (2006). Handbook of enology: The chemistry of wine stabilization and treatments, second edition. Vol. 2. Chichester: John Wiley & Sons Ltd.

16. Sprenger S, Hirn S, Dietrich H, Will F (2015). Metatartaric acid: Physiochemical characterization and analytical detection in wines and grape juices. Eur Food Res Technol. 241:785–91. doi:10.1007/s00217–015–2503–1.

17. Underhill FP, Leonard CS, Gross EG, Jaleski TC (1931). Studies on the metabolism of tartrates II. The behavior of tartrate in the organism of the rabbit, dog, rat and guinea pig. J Pharmacol Exp Ther. 43(2):359–80.

3.1.7 Tamarind seed polysaccharide

Explanation

Tamarind seed polysaccharide (CAS No. 39386-78-2) is produced from the hulled seeds of *Tamarindus indica* Linne. Tamarind seed polysaccharide is a xyloglucan. Xyloglucans are a type of dietary fibre naturally present in the cell wall of plants, which are abundant in rice, vegetables and fruits (Shibuya & Iwasaki, 1978; Kato & Matsukura, 1994; Kato, 1995; Kato, Ito & Watanabe, 2001). Tamarind seed polysaccharide is permitted for use as a thickener, stabilizer, emulsifier and gelling agent in a variety of food products in China, Japan, the Republic of Korea and the USA.

Tamarind seed polysaccharide has not been previously evaluated by JECFA. The Committee evaluated tamarind seed polysaccharide at the request of the Forty-eighth Session of the CCFA (FAO/WHO, 2016).

A toxicological dossier for tamarind seed polysaccharide was submitted. A comprehensive literature search was also conducted. None of the records retrieved added to the toxicological data submitted to the Committee for this meeting.

To address any data gaps, the Committee also considered safety data on other polysaccharide-based gums on the basis of their similar general structure, chemical and functional properties, technical uses, lack of absorption as intact substances and metabolism to normal dietary constituents (e.g. short-chain fatty acids) as a result of microbial fermentation in the large intestine.

Chemical and technical considerations

The tamarind tree is a large evergreen widely distributed in subtropical and tropical zones (Williams, 2006). *T. indica* L. is a monotypic genus and belongs to the subfamily Caesalpinioideae of the family Leguminosae (Fabaceae). The seeds of the tamarind fruit are smooth, glossy, flattened and oblong-shaped (Duke, 1981). Tamarind seed polysaccharide is also known as tamarind seed gum, tamarind gum, tamarind xyloglucan, tamarind seed xyloglucan and tamarind galactoxyloglucan.

Every part of the *T. indica* L. tree is used as food or in traditional medicine in most tropical countries (De Caluwé, Halamová & Van Damme,

2010). Traditional uses in food rely on the aroma and flavouring properties of the tamarind fruit, in its fresh or dried form. It is also used in herbal medicinal therapies (Williams, 2006).

Tamarind seed polysaccharide is produced from tamarind seeds that are sieved and toasted to remove the black testa (seed coat). The light brown tamarind kernel obtained is then pulverized and sieved to obtain tamarind kernel powder. The kernels contain 65–72% carbohydrate (polysaccharide and free sugars), 15–23% protein, 4–7% fat, 2–3% ash and 0.7–8% crude fibre, reported on a dry matter basis (Duke, 1981). The tamarind kernel powder is treated with methanol, and the pH is adjusted during treatment; this is followed by centrifugation to physically separate the insoluble tamarind seed polysaccharide from the supernatant, which contains the protein, fat and minerals. The polysaccharide is dried, pulverized, sieved and mixed with bulking agents to standardize the product. Depending on the pH treatment, downstream filtration, and acid or alkali treatment, products differing by viscosity can be manufactured.

Tamarind seed polysaccharide is composed of a linear chain of D -glucose units linked by β(1–4) glycosidic bonds. Single D-xylose units are attached to about 75% of these D-glucose units via α(1–6) bonds. Single D-galactose units are attached to some of the D-xylose units through β(1–2) bonds. The molar ratio of glucose:xylose:galactose is about 4:3:1 (Gidley et al., 1991). The tendency of xyloglucans to self-associate gives rise to a wide range of reported molecular weights (400–6000 kDa) (Nishinari et al., 2009).

Biochemical aspects

Absorption, distribution, metabolism or excretion data were not available on tamarind seed polysaccharide. Based on its size and chemical composition, tamarind seed polysaccharide, like other dietary fibres, is not expected to be absorbed intact or digested in the gastrointestinal tract (Cummings & Englyst, 1987). Based on its chemical composition, tamarind seed polysaccharide is expected to be enzymatically degraded and fermented by intestinal bacteria in the large intestine. The fermentation process would yield hydrogen gas, carbon dioxide and short-chain fatty acids, which could be absorbed and metabolized. It has been estimated that more than 75% of tamarind seed polysaccharide is fermented (Ministry of Health, Labour and Welfare, 2003). This extensive fermentation process is similar to that for other nondigestive polysaccharides, such as carob bean gum, cassia gum and tara gum.

Evidence supporting such a fermentation process includes the results of a 14-day dietary study in rats, which showed that oligosaccharides of tamarind seed polysaccharide generate short-chain fatty acids (specifically, lactic acid, propionic acid and butyric acid) in the caeca of test animals in greater amounts than in control rats fed a non-fibre diet (Ebihara & Nakamoto, 1998). In vitro

studies demonstrated that human microflora can also degrade and ferment tamarind seed polysaccharide (Hartemink et al., 1996). Specific bacteria that colonize the large intestine in humans are capable of enzymatic hydrolysis of the glucan backbone of xyloglucans, which would lead to fermentation (Hartemink et al., 1996; Larsbrink et al., 2014).

Toxicological studies

All toxicological tests were conducted using a commercial product in which the purity of the tamarind seed polysaccharide was between 80% and 85%. The remaining 15–20% included water, carbohydrates, protein and fat, which are normal dietary constituents that are not expected to pose a toxicological hazard. Tamarind seed polysaccharide is of low acute oral toxicity in mice and rats. The LD_{50} in each of these species was greater than 5000 mg/kg bw (4000 mg/kg bw when corrected for purity).

No toxicity was observed in a 13-week study in mice at concentrations of up to 50 000 mg/kg feed (equal to 8200 mg/kg bw per day, or 6642 mg/kg bw per day when corrected for purity) (Sano et al., 1996). There were no toxicologically relevant effects in a 4-week dietary study of tamarind seed polysaccharide in rats at concentrations up to 120 000 mg/kg feed (equal to 10 597 mg/kg bw per day, or 9113 mg/kg bw per day when corrected for purity) (Heimbach et al., 2013; Koetzner, 2013).

Similarly, no toxicologically relevant effects, including treatment-related tumours, were observed in a 78-week study in mice at concentrations of up to 50 000 mg/kg feed (equal to 6658 mg/kg bw per day, or 5380 mg/kg bw per day when corrected for purity) (Sano et al., 1996). No treatment-related toxicity, including tumours, was observed in a 104-week study in rats at concentrations of up to 120 000 mg/kg feed (equal to 5150 mg/kg bw per day, or 4161 mg/kg bw per day when corrected for purity) (Iida et al., 1978). The highest doses tested in these toxicity studies routinely equalled or exceeded the recommended dose limit of 5% of the diet for rodent toxicity studies.

The Committee concluded that the pivotal study was the 104-week study in rats (Iida et al., 1978). This was a well-conducted study performed before the implementation of GLP. The NOAEL was 5150 mg/kg bw per day (corrected to 4161 mg/kg bw per day for purity), the highest dose tested.

Tamarind seed polysaccharide tested negative in bacterial reverse mutation assays and in an in vitro chromosomal aberration assay. Despite the limitations of some of these assays (due to the poor solubility of the test substance at higher concentrations), based on the absence of chemical structural alerts and negative results, the Committee concluded that for tamarind seed polysaccharide, there was no concern with respect to genotoxicity.

No reproductive or developmental toxicity studies were conducted with tamarind seed polysaccharide. The Committee noted that histopathological analysis of reproductive organs from long-term feeding studies in mice and rats did not identify any effects on reproductive tissues. The Committee also noted that reproductive and developmental toxicity studies on other polysaccharide gums previously evaluated by the Committee did not raise concerns for reproductive or developmental effects. For example, when cassia gum was assessed in a two-generation reproductive toxicity study in rats, it was shown not to cause reproductive toxicity at 50 000 mg/kg feed (equal to 5280 mg/kg bw per day), the highest concentration tested. In a developmental toxicity study in rats, cassia gum did not cause embryotoxicity or teratogenicity at 1000 mg/kg bw per day, the highest dose tested. In a developmental toxicity study in rabbits, cassia gum did not cause any adverse effects on dams or numbers of implantations, postimplantation losses or fetal defects at 1000 mg/kg bw per day, the highest dose tested.

Based on the absence of histopathological effects on reproductive tissues in long-term rodent studies, the lack of absorption of intact tamarind seed polysaccharide, the degradation and fermentation of tamarind seed polysaccharide into normal dietary constituents, and the absence of reproductive or developmental toxicity observed with other polysaccharide gums, the Committee concluded that tamarind seed polysaccharide would be unlikely to pose a concern with respect to reproductive or developmental toxicity.

Observations in humans

No reports were found on food allergies or food intolerance to tamarind seed polysaccharide, despite its long-term use in several countries.

Assessment of dietary exposure

The Committee received an assessment of dietary exposure to tamarind seed polysaccharide from one sponsor in response to the call for data.

Two national estimates of dietary exposure to tamarind seed polysaccharide were included in the sponsor's submission and reviewed by the Committee: from Japan and the USA. These estimates of dietary exposure to tamarind seed polysaccharide were made by combining maximum use levels (assuming 85% polysaccharide in the commercial product) with 2014 food consumption data from the Japanese National Health and Nutrition Survey or with 2003–2006 food consumption data from USA National Health and Nutrition Examination Surveys. The estimated mean dietary exposure to tamarind seed polysaccharide ranged from 31 to 38 mg/kg bw per day, with the 90th percentile exposures up to 77 mg/kg bw per day. These estimates are conservative, in that it has been assumed that all products that might contain tamarind seed

polysaccharide would contain the substance at the indicated maximum use levels. Tamarind seed polysaccharide would be likely to substitute for other gums.

The Committee concluded that the estimated dietary exposure of 75 mg/kg bw per day was suitable for use in this safety assessment.

Evaluation

The Committee established an ADI "not specified" for tamarind seed polysaccharide. This ADI was based on the absence of toxicity in repeated-dose animal studies of tamarind seed polysaccharide. These included long-term rodent studies in which mice were fed up to 6658 mg/kg bw per day (corrected to 5380 mg/kg bw per day for purity) and rats up to 5150 mg/kg bw per day (corrected to 4161 mg/kg bw per day for purity). In addition, there was no concern regarding genotoxicity. Reproductive toxicity and developmental toxicity were not considered a concern based on the lack of absorption of intact polysaccharide, the degradation and fermentation of tamarind seed polysaccharide into normal dietary constituents and the absence of reproductive and developmental effects in other polysaccharide gums.

The estimated dietary exposure based on proposed uses and use levels was 75 mg/kg bw per day. The Committee concluded that this does not present a health concern.

A toxicological and dietary exposure monograph was prepared.

A new specifications monograph and a Chemical and Technical Assessment were prepared.

References

1. Cummings JH, Englyst HN (1987). Fermentation in the human large intestine and the available substrates. Am J Clin Nutr. 45:1243–55.
2. De Caluwé E, Halamová K, Van Damme P (2010). Tamarindus indica L.– A review of traditional uses, phytochemistry and pharmacology. Afrika Focus. 23(1):53–83.
3. Duke JA (1981). Handbook of legumes of world economic importance. New York: Plenum Press; 228–30.
4. Ebihara K, Nakamoto Y (1998). Comparative effect of water-soluble and –insoluble dietary fiber on bowel function in rats fed a liquid elemental diet. Nutr Res. 18(5):883–91.
5. FAO/WHO (2016). 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 Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission (REP 16/FA).
6. Gidley MJ, Lillford PJ, Rowlands DW, Lang P, Dentini M, Crescenzi V et al. (1991). Structure and solution properties of tamarind-seed polysaccharide. Carbohydr Res. 214(2):299–314.
7. Hartemink R, Van Laere KMJ, Mertens AKC, Rombouts FM (1996). Fermentation of xyloglucan by intestinal bacteria. Anaerobe. 2(4):223–30.

8. Heimbach JT, Egawa H, Marone PA, Bauter MR, Kennepohl E (2013). Tamarind seed polysaccharide: A 28-day dietary study in Sprague-Dawley rats. Int J Toxicol. 32(3):198–208.

9. Iida M, Matsunaga Y, Matsuoka N, Abe M, Ohnishi K, Tatsumi H (1978). [Two years feeding toxicity study of tamarind seed polysaccharide in rats]. J Toxicol Sci. 3(2):163–92 (in Japanese).

10. Kato Y (1995). [Carbohydrate composition of major root vegetables]. Bull Fac Educ Hirosaki Univ. 74:37–47 (in Japanese).

11. Kato Y, Matsukura J (1994). [Carbohydrate composition of major leaf vegetables]. Bull Fac Educ Hirosaki Univ. 71:61–71 (in Japanese).

12. Kato Y, Ito S, Watanabe T (2001). [Polysaccharide composition of the water-insoluble dietary fibers from some fruit]. Bull Fac Educ Hirosaki Univ. 86:91–6 (in Japanese).

13. Koetzner L (2013). Glyloid 2A: A 28-day dietary study in rats. Study no. 34510. Unpublished study conducted by Product Safety Labs, Dayton, New Jersey, USA. Submitted to WHO by Dainippon Pharmaceutical Co. Ltd.

14. Larsbrink J, Rogers TE, Hemsworth GR, McKee LS, Tauzin AS, Spadiut O et al. (2014). A discrete genetic locus confers xyloglucan metabolism in select human gut. Bacterioidetes. Nature. 506:498–502.

15. Ministry of Health, Labour and Welfare (2003). Partial revision of the analytical methods for nutrition ingredient in the Nutrition Labeling Standards. Government of Japan MHLW. Shou-shin notification no. 0217002.

16. Nishinari K, Takemasa M, Yamatoya K, Shirakawa M (2009). Xyloglucan. In: Phillips GO, Williams PA, editors. Handbook of hydrocolloids, second edition. New York: CRC Press; 535–66.

17. Sano M, Miyata E, Tamano S, Hagiwara A, Ito N, Shirai T (1996). Lack of carcinogenicity of tamarind seed polysaccharide in B6C3F1 mice. Food Chem Toxicol. 34:463–7.

18. Shibuya N, Iwasaki T (1978). Polysaccharides and glycoproteins in the rice endosperm cell wall. Agric Biol Chem. 42:2259–66.

19. Williams JT (2006). Introduction, taxonomy, description and distribution. In: Williams JT, Smith RW, Haq N, Dunsiger Z, editors. Tamarind, Tamarindus indica L. Southampton: Southampton Centre for Underutilised Crops: 1–22.

3.1.8 Tannins

Explanation

Oenological tannins, which are derived from a variety of natural sources, including grape seeds and skins, stems and stalks, nutgalls and tannin-rich wood, are additives or processing aids used in wine. Tannins are also found in numerous other natural food items. Tannins are an extensive group of compounds that can be broadly divided into two main categories, condensed and hydrolysable tannins, with complex tannins being a mixture of the two. Condensed tannins are formed from polymerization reactions of leucocyanidins and flavan-3-ols and are largely derived commercially from grapes; the flavan-3-ol monomeric subunits include (+)-catechin and (−)-epicatechin and their gallates. Condensed tannins are divided into pro-anthocyanidins and profisetidin and are not susceptible to

hydrolytic cleavage. Hydrolysable tannins (gallotannins and ellagitannins) are largely derived from heartwood. They may undergo hydrolysis to yield gallic acid and ellagic acid and related saccharides.

Oenological tannins are used in winemaking to clarify musts and wines, prevent protein and metal–protein haze, stabilize red wine colour, prevent oxidization, inhibit the activity of the enzyme laccase, and improve the astringency and mouthfeel properties of wine.

The Committee evaluated tannic acid at its fifth, tenth, fourteenth, thirty-first and thirty-fifth meetings (Annex 1, references 5, 13, 22, *77* and *88*). The Committee revised metals and arsenic specifications for tannic acid at its sixty-third meeting (Annex 1, reference *173*). The Committee evaluated grape skin extract at its twenty-eighth meeting (Annex 1, reference *66*). The Committee revised metals and arsenic specifications for grape skin extract at its fifty-ninth meeting (Annex 1, reference *160*).

Anthocyanins were evaluated by the Committee at its twenty-sixth meeting, when an ADI of 0–2.5 mg/kg bw was established (Annex 1, reference *59*). This was based on a level of 7.5% grape skin extract in the diet, which caused no toxicological effect in a two-generation reproductive toxicity study in rats (Cox & Babish, 1978; Becci et al., 1983). Tannic acid (the major hydrolysable gallotannin) was evaluated by the Committee at its tenth, fourteenth, thirty-first and thirty-fifth meetings (Annex 1, references *13*, *22*, *77* and *88*). At its thirty-first meeting, the Committee established a temporary ADI "not specified" for tannic acid used as a filtering aid in food (Annex 1, reference *77*), and the temporary status was removed at the thirty-fifth meeting (Annex 1, reference *88*). At the fourteenth meeting, a temporary ADI of 0–0.6 mg/kg bw per day was established for tannins derived from Peruvian tara, and a temporary ADI of 0–0.3 mg/kg bw per day was established for tannins derived from Turkish Aleppo, Chinese tara and Sicilian sumac.

Tannins were evaluated based on a request from the Forty-eighth Session of the CCFA (FAO/WHO, 2016). The only submission in response to the JECFA call for data related to the use of tannins as a food additive in winemaking and consisted of a literature review rather than specifically commissioned studies. As there were already specifications for tannic acid, the submission largely covered the condensed tannins and the hydrolysable ellagitannins, although some data on tannic acid were also included. Additional studies related to the sources of tannins used in winemaking were identified in a literature search conducted by the Committee.

Chemical and technical considerations

Oenological tannins are manufactured from raw materials such as nutgalls, tara (*Caesalpinia spinosa*) pods, heartwoods (chestnut, oak, exotic wood such as

quebracho wood), other plants such as myrobolan fruits, or grape seeds and skins, stems and stalks. Most tannins available in commerce are extracted with water, steam, ethanol, ethyl acetate or acetone (or a mixture of these solvents), dried and milled. Different products may have undergone hydrolysis to varying degrees, pH and colour adjustment, sulfite addition and spray-drying. The composition of oenological tannins varies considerably depending on the botanical source of the raw material, extraction method, processing, purification and degree of polymerization (or the number of flavan-3-ol subunits).

Biochemical aspects

Tannins are high molecular weight carbohydrate polymers that are poorly absorbed; they may be broken down by gut microorganisms to smaller oligomers and monomers, which are more easily absorbed. Smaller oligomers are present in some of the tannin sources studied and may also be present in the oenological tannins, but the extent of this is unknown. Data on the absorption of proanthocyanidin polymers, procyanidin dimers and ellagitannins in laboratory species and/or humans were available. However, the relevance of these data to the oenological tannins used commercially is unclear, both because some of the tannin sources used are less well characterized (e.g. oak, chestnut, exotic woods) and because the composition of blends of oenological tannins that may be used is unknown. Both condensed and hydrolysable tannins are broken down by gut microflora into smaller units (monomers, dimers and oligomers), with further metabolism and some degree of conjugation then occurring. The available literature indicates that there is significant interindividual variation in metabolism of tannins in both humans and laboratory animal species. Data on tissue distribution and excretion for both groups of tannins are limited and indicate that distribution into specific tissues or organs may occur. Excretion of tannins and their metabolites occurs via the bile and/or the urine.

Toxicological studies

Data on acute, short-term and long-term toxicity, genotoxicity and reproductive toxicity were available for different tannins. For the condensed tannins, the majority of data relate to grape seed and grape skin extracts, whereas for the hydrolysable tannins, the majority of the data relate to tannic acid and pomegranate extract (a source of ellagitannins). However, the toxicological data available for both classes of tannins are limited, with information on long-term toxicity and reproductive and developmental toxicity particularly lacking. There are even fewer toxicological data specific to sources such as oak and chestnut tannins. A number of studies relate to proposed beneficial effects, where the tannins were used to prevent oxidative or genotoxic damage from toxicants; only limited information can be obtained from these studies. The rationale for

the inclusion of certain studies (notably on the hydrolysable ellagitannins, such as would be present in pomegranate extract) in the submission and how these compare to the oenological tannins as used are not clear.

The toxicity of condensed and hydrolysable tannins is low. Tannic acid is known to be hepatotoxic at high oral doses (1.7 g/kg bw in a 90-day rat study [Niho et al., 2001] and ≥2 g/kg bw in single-dose studies in rats [Boyd, Bereczky & Godi, 1965; Zhu, Filippich & Alsalam, 2001]), but there is no evidence of this occurring at lower doses. There are in vitro and in vivo genotoxicity studies available for both condensed and hydrolysable tannins; the results are largely negative, but some positive findings were reported. However, the significance of these findings relative to the oenological tannins used commercially is uncertain.

Observations in humans

Some of the tannins have been tested in human volunteer studies generally designed to assess their potential beneficial effects when used as food supplements. Some relevant information, particularly on the effects on haematology and serum biochemistry, can be obtained from these studies; there are no suggestions of adverse effects. However, how the tannins assessed in these studies compare to the oenological tannins used commercially is unclear.

Assessment of dietary exposure

The sponsor requested the use of (oenological) tannins in wine at use levels of 50–100 mg/L. To assess the potential exposure to tannins added to wine at these use levels, the Committee prepared international estimates of dietary exposure to tannins added to wine using wine consumption levels from the CIFOCOss database (food categories 14.2.3.1 "Still grape wine" and 14.2.3.3 "Fortified grape wine, grape liquor wine and sweet grape wine"). The mean dietary exposure to added tannins in wine at a use level of 75 mg/L ranged from 0.0005 to 0.12 mg/kg bw per day in adults. The highest exposure in adult consumers of wine (95th percentile combined with the maximum use level of 100 mg/L) was 0.77 mg/kg bw per day.

Tannins also occur naturally in many foods, including fruits, vegetables, cereals, beans, nuts, cocoa beans, tea and beer, as well as wine (Gu et al., 2004; USDA, 2004; Prior & Gu, 2005; Serrano et al., 2009). In several studies, the exposures to two types of tannins through the regular diet were reported: proanthocyanidins, the major tannins ingested in the western diet (Prior & Gu, 2005), and ellagitannins. Exposure estimates were reported for Finland (Ovaskainen et al., 2008), France (Pérez-Jiménez et al., 2011), Germany (Radtke, Linseisen & Wolfram, 1998), Spain (Saura-Calixtoa, Serrano & Goñi, 2007; Tresserra-Rimbau et al., 2013) and the USA (Gu et al., 2004). Mean exposures to proanthocyanidins in adults ranged from 71 mg/day in the USA to 450 mg/day

in Spain. The main contributors to the exposure to proanthocyanidins in these studies were red wine, fruit (berries, apples, oranges, peaches) and chocolate products. Only very limited exposure data on ellagitannins were available: from Germany (Radtke, Linseisen & Wolfram, 1998) and Finland (Ovaskainen et al., 2008). Reported mean exposures in adults were 5.2 and 12 mg/day, respectively. Assuming a body weight of 60 kg, the reported mean exposures to proanthocyanidins in adults would range from 1 to 8 mg/kg bw per day. These exposure levels from the diet are 1.3–10 times higher than the highest exposure in adults consuming wine (0.77 mg/kg bw per day) and 8–70 times higher than exposure in average consumers of wine. The Committee noted that because the exact composition of oenological tannins is unknown, these comparisons are to provide context only.

Overall, the exposure to tannins added to wine is expected to be lower than estimated by the Committee: tannins added to wine will be partially removed, as they precipitate with a proteinaceous matter that is subsequently removed by decantation or filtration. Furthermore, the addition of tannins to wine is technologically self-limiting, because the wine may become unacceptably astringent at high tannin levels.

Evaluation

The available data do not provide clear information on which tannin sources and individual tannin compounds are present in commercially used oenological tannins and thus how the oenological tannins would compare to the tannins used in the submitted studies. Therefore, it is not possible to establish which studies are relevant and consequently the extent of the data gaps. Some of the oenological tannins (e.g. grape seed and skin extracts) are better characterized than others (e.g. oak and chestnut).

Many of the available literature studies use a test substance that is derived from the same tannin source, such as grape skin extract, but this may have been an extract prepared in the laboratory or prepared commercially for use as a food additive or in food supplements: it is unclear how the compositions of these preparations compare to each other or to the same source when present in commercial oenological tannins.

The information on biochemical aspects is incomplete, with the implications of repeated dosing on absorption, tissue distribution and interindividual variation needing consideration. In general, there are also few data available on reproductive and developmental toxicity and/or long-term toxicity for some or all of the tannins.

In the absence of specifications and identification of the products in commerce, the Committee concluded that it is not possible to evaluate tannins used in winemaking.

A toxicological and dietary exposure monograph was not prepared.

No specifications monograph was prepared.

The Committee assessed the information received and concluded that there were insufficient data and information to prepare specifications for oenological tannins. The Committee requires data for the characterization of the products in commerce to be able to complete specifications for oenological tannins used as an antioxidant, colour retention agent and stabilizer in wine. The required information includes a detailed description of the manufacturing processes and thorough chemical characterization of the commercial products made from different botanical sources.

The following information is required:

- Composition of tannins derived from the full range of raw materials as well as the processes used in their manufacture;
- Validated analytical method(s) and relevant quality control data;
- Analytical data from five batches of each commercial product including information related to impurities such as gums, resinous substances, residual solvents, sulfur dioxide content and metallic impurities (arsenic, lead, iron, cadmium and mercury);
- Solubility of the products in commerce, according to JECFA terminology; and
- Use levels, natural occurrence and food products in which tannins are used.

Submitters are encouraged to offer a rationale for a single specifications monograph for oenological tannins covering all products or individual monographs.

References

1. Becci PJ, Hess FG, Babish JG, Gallo MA, Voss KA (1983). Reproduction study of grape colour extract in rats. Food Chem Toxicol. 21:79–83.

2. Boyd EM, Bereczky K, Godi I (1965). The acute toxicity of tannic acid administered intragastrically. Can Med Assoc J. 92:1292–7.

3. Cox GE, Babish JC (1978). Evaluation of the safety of dietary administration of special grape color powder (type BW-AT) on reproduction, lactation and maturation when fed to Sprague-Dawley rats. Unpublished report no. 5417 by Food and Drug Research Laboratories, Inc., submitted to WHO by USFDA.

4. FAO/WHO (2016). 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 Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission (REP 16/FA).

5. Gu L, Kelm MA, Hammerstone JF, Beecher G, Holden J, Haytowitz D et al. (2004). Concentrations of proanthocyanidins in common foods and estimations of normal consumption. J Nutr. 134:613–7.

6. Niho N, Shibutani M, Tamura T, Toyoda K, Uneyama C, Takahasi et al. (2001). Subchronic toxicity study of gallic acid by oral administration in F344 rats. Food Chem Toxicol. 39:1063–70.

7. Ovaskainen ML, Törrönen R, Koponen JM, Sinkko H, Hellström J, Reinivuo H et al. (2008). Dietary intake and major food sources of polyphenols in Finnish adults. J Nutr. 138(3):562–6. Pérez-Jiménez J, Fezeu L, Touvier M, Arnault N, Manach C, Hercberg S et al. (2011). Dietary intake of 337 polyphenols in French adults. Am J Clin Nutr. 93(6):1220–8. doi:10.3945/ajcn.110.007096.

8. Prior RL, Gu L (2005). Occurrence and biological significance of proanthocyanidins in the American diet. Phytochemistry. 66:2264–80. doi:10.1016/j.phytochem.2005.03.025.

9. Radtke J, Linseisen J, Wolfram G (1998). [Phenolic acid intake of adults in a Bavarian subgroup of the national food consumption survey]. Z Ernährungswiss. 37:190–7.

10. Saura-Calixtoa F, Serranob J, Goñi I (2007). Intake and bioaccessibility of total polyphenols in a whole diet. Food Chem. 101:492–501. doi:10.1016/j.foodchem.2006.02.006.

11. Serrano J, Puupponen-Pimiä R, Dauer A, Aura AM, Saura-Calixto F (2009). Tannins: Current knowledge of food sources, intake, bioavailability and biological effects. Mol Nutr Food Res. 53(Suppl. 2):S310–29. doi:10.1002/mnfr.200900039.

12. Tresserra-Rimbau A, Medina-Remón A, Pérez-Jiménez J, Martínez-González MA, Covas MI, Corella D et al. (2013). Dietary intake and major food sources of polyphenols in a Spanish population at high cardiovascular risk: The PREDIMED study. Nutr Metab Cardiovasc Dis. 23:953–9. doi:10.1016/j.numecd.2012.10.008.

13. USDA (2004). USDA Database for the proanthocyanidin content of selected foods. Beltsville (MD): United States Department of Agriculture.

14. Zhu J, Filippich LJ, Alsalam MT (1992). Tannic acid intoxication in sheep and mice. Res Vet Sci. 53:280–92.

3.1.9 Yeast extracts containing mannoproteins

Explanation

Yeast extracts containing mannoproteins are used as food additives in winemaking. Yeast mannoproteins are extracted from purified yeast (*Saccharomyces cerevisiae*) cell walls by enzymatic treatment with β-glucosidase or by physicochemical extraction with thermal treatment. Yeast mannoproteins are galactomannans consisting almost exclusively of mannose units bound to proteins or peptides.

The name was changed from "yeast mannoproteins" to "yeast extracts containing mannoproteins" because the name "yeast mannoproteins" was not adequately descriptive. The products in commerce are extracts containing yeast components and mannoproteins, and not pure mannoproteins. Yeast extracts containing mannoproteins have not been previously evaluated by the Committee. The compounds were evaluated at the present meeting at the request of the Forty-eighth Session of the CCFA (FAO/WHO, 2016). The JECFA call asked for data

on yeast mannoproteins in general; however, the only data that were submitted related to their use as a food additive in winemaking.

Wine contains significant concentrations of tartrates that can crystallize and precipitate during storage, resulting in unwanted sediment. Wine also contains small amounts of protein, which can produce a haze. Although yeast mannoproteins occur naturally in wine due to yeast fermentation, they are also added to inhibit the crystallization of tartrates and stabilize the proteins in the wine after bottling and during storage.

Yeast mannoproteins are approved for treatment of wine in Argentina, Australia, Canada, the European Union (Commission Regulation (EC) No. 2165/2005), New Zealand and the USA.

The sponsor submitted a dossier summarizing technological, toxicological and dietary exposure information relevant to the evaluation of yeast mannoproteins from *S. cerevisiae*. In addition, a literature search for toxicity data performed using multiple databases and search terms resulted in approximately 20 other potentially relevant papers. However, because few toxicological studies were available for yeast mannoproteins, relevant studies with *S. cerevisiae*, its constituents or substances derived from its fermentation were included in the assessment.

Chemical and technical considerations

Mannoproteins represent a large group of natural compounds from yeast (*S. cerevisiae*) in which polysaccharide chains are bound to proteins and peptides by covalent and non-covalent linkages (i.e. ionic interactions). The structures and molecular weights of mannoproteins vary, depending on the degree and type of glycosylation. The polysaccharide chains consist almost exclusively of mannose units linked by α-links forming a long α-1→6 linked backbone containing short α-1→2 and α-1→3 linked side-chains. Several of the side-chains may have phosphodiester linkages to other mannosyl residues. Yeast mannoproteins are extracted from purified yeast cell walls by enzymatic extraction using glucan 1,3-β-glucosidase (EC 3.2.1.58) or by thermal treatment. The enzyme hydrolyses the yeast cell wall, allowing the mannoproteins to be solubilized. The thermal treatment breaks the links with β-glucans in the cell wall to release the mannoproteins. The mannoproteins thus solubilized by either treatment are then separated from the insoluble cell wall material, concentrated and micro-filtered or ultra-filtered. The mannoproteins have molecular weights ranging from 20 kDa to more than 450 kDa.

There was limited information available to the Committee to fully characterize the yeast mannoprotein products in products of commerce. Information and data about the chemical composition of the range of commercial yeast mannoprotein products are required. There are also limited data available on the levels of yeast mannoproteins in wine. Wine contains yeast mannoproteins from the fermentation process as well as those added for the purpose of

precipitating tartrates. This results in potential levels higher than 400 mg/L of yeast mannoproteins in the wine.

Biochemical aspects

No relevant absorption, distribution, metabolism or excretion studies were available for yeast mannoproteins. The Committee assumed that mannoproteins extracted from *S. cerevisiae* in the test compound will behave similarly to those resulting from dietary exposure to the intact yeast or to other glucomannans consumed as part of a regular diet. Once mannoproteins have been hydrolysed by intestinal enzymes, the carbohydrate moiety can be fermented by intestinal microflora in the large intestine into, among others, organic acids or alcohols (den Besten et al., 2013 a,b; Bagenholm et al., 2017).

In a study using immortalized human hepatocytes (Fa2N-4 cells), a fermentation product of *S. cerevisiae* did not induce cytochrome P450 (CYP) CYP1A2 or CYP3A4 messenger ribonucleic acid or enzymatic activity and did not interfere with the induction of CYP1A2 or CYP3A4 by omeprazole or rifampin (also known as rifampicin), respectively (Schauss et al., 2012). The test article used in this study was described as the product of a proprietary fermentation process using *S. cerevisiae*, involving "both a unique substrate and a stress process". The test article, hereafter referred to as "yeast fermentate preparation", was also tested in several toxicology studies. The yeast fermentate preparation is reported to contain cell wall components, including mannoproteins, components from the medium, fermentative by-products and stress-induced metabolites. However, a more complete chemical characterization of the test article was not available.

Toxicological studies

In male rats given a daily dose of 10^8 viable cells or colony-forming units of *S. cerevisiae* RC016 by oral gavage for 60 days, no treatment-related effects were reported (González Pereyra et al., 2014).

In a 90-day study, groups of male and female rats were given 0, 30, 200 or 1500 mg/kg bw per day of a suspension of yeast fermentate preparation in water containing 1% methylcellulose. No deaths occurred, and no treatment-related changes in any of the parameters assessed at any dose were observed (Schauss et al., 2012).

Schauss et al. (2012) reported a chronic toxicity study in male and female rats administered 0, 20, 200 or 800 mg/kg bw per day of a suspension of yeast fermentate preparation in water containing 1% methylcellulose. No treatment-related or clinically relevant findings were reported in any of the parameters assessed at any dose.

A yeast fermentate preparation was negative in a bacterial reverse mutation assay and in a mouse lymphoma cell mutagenicity test (Schauss et al.,

2012). Bone marrow micronucleus and comet assays were negative in male rats given 10^8 viable cells or colony-forming units of *S. cerevisiae* RC016 daily for 60 days by oral gavage (González Pereyra et al., 2014).

No data were available regarding the carcinogenicity and reproductive or developmental toxicity of material relevant to yeast extracts containing mannoproteins.

The only study available with yeast extracts containing mannoproteins gave a negative result in a dermal sensitization study conducted on albino guinea-pigs (Richeux, 2002).

Owing to the high content of mannose in yeast, the Committee assumed that yeast mannoproteins, like other galactomannans, can interact with mannose receptors (Tizard et al., 1989). Binding of mannosylated proteins to mannose receptors is involved in various physiological mechanisms, including innate and specific immunity. The consequences of increased binding of mannoproteins to mannose receptors and the relevance of such data are still a matter of research.

Observations in humans

Yeast fermentate preparation from *S. cerevisiae* was not mitogenic in human peripheral lymphocytes (Schauss et al., 2012).

Bansal, Tadros & Bansal (2017) reported one case of allergy to beer, wine and cider resulting from immunoglobulin E reactivity to yeasts and moulds.

Assessment of dietary exposure

Yeast extracts containing mannoproteins are proposed for use at a recommended use level of 200 mg/L and at a maximum level of 400 mg/L in food category 14.2.3 "Grape wines" and its subcategories within the GSFA. Yeast mannoproteins also occur naturally in wine, as well as in other foods including bread, pastries, beer and yeast extracts, and in food supplements. The Committee evaluated the sponsor's submission and prepared international estimates of dietary exposure to yeast mannoproteins using the CIFOCOss database in combination with the recommended and maximum use levels in wine and the background occurrence of yeast mannoproteins in wine, bread, pastries and beer. No consumption data on yeast extracts and yeast-containing food supplements were available in CIFOCOss. The dietary exposure was calculated using datasets in the CIFOCOss that were related to food consumption data for adolescents (10–18 years), adults (18+ years) and the general population (ages not specified), assuming that 100% of the yeast extract was mannoproteins.

The mean background exposure to yeast mannoproteins ranged from 0.1 to 21 mg/kg bw per day. In consumers with high consumption of wine, the background exposure ranged from 2.5 to 21 mg/kg bw per day. The highest background exposures were calculated for adolescents. Addition of yeast extracts

containing mannoproteins to wine at the recommended level resulted in an increase in the mean dietary exposure to yeast mannoproteins in the datasets of less than 5% (<0.1–4.2%), resulting in a range of dietary exposure of 0.4–21 mg/kg bw per day. For consumers with high consumption of wine, the addition of yeast extracts containing mannoproteins to wine at the maximum level resulted in an increase of dietary exposure of, on average, 20%. The resulting high estimates of dietary exposure were 4.3–21 mg/kg bw per day. Dietary exposure to yeast mannoproteins was mainly (at least 90% in almost all datasets) determined by bread and pastries, due to both high consumption and a high concentration level. The additional dietary exposure to yeast mannoproteins via the consumption of yeast extract, based on FSANZ data (FSANZ, 2008), was estimated to be about 3 mg/kg bw per day.

Evaluation

The Committee noted that very few toxicity studies were available for the range of yeast extracts containing mannoproteins on the market. However, consumers are exposed to yeast mannoproteins from *S. cerevisiae* present in wine as well as in other fermented foods, including bread, pastries, beer and yeast extracts, and in food supplements. Therefore, the Committee considered that it was possible to use the available information relative to *S. cerevisiae* and its constituents for this evaluation. No indication for toxicity was identified from the available information, including the toxicological studies on one product that is poorly characterized (yeast fermentate preparation from *S. cerevisiae*). However, there were no data on reproductive and developmental toxicity or carcinogenicity for any relevant yeast preparation.

In addition to the natural presence of yeast mannoproteins in wine and the long history of consumption of yeast products in common foods, the Committee considered that the tentative product specifications for yeast extracts containing mannoproteins indicate that these do not contain chemical residues or microbiological contaminants of concern. In addition, the Committee estimated that the exposure to yeast mannoproteins due to the addition of yeast extracts containing mannoproteins to wine at the maximum level of 400 mg/L would result, on average, in a 20% increase in dietary exposure compared to the background exposure through the regular diet of 0.4–21 mg/kg bw per day, primarily driven by bread and pastries. These conservative dietary exposure estimates are based on the assumption that 100% of the yeast extracts containing mannoproteins is mannoproteins.

In considering the data and information regarding yeast and yeast-derived products, the Committee concluded that it is unlikely that there would be a health concern for the use of yeast extracts containing mannoproteins as a

food additive for oenological uses at maximum use levels up to 400 mg/L for the stabilization of wine.

The Committee noted that any change in the uses and/or use levels of yeast extracts containing mannoproteins as a food additive will require a new evaluation.

A toxicological and dietary exposure monograph was prepared.

A new tentative specifications monograph and a Chemical and Technical Assessment were prepared.

In order to remove the tentative designation of the specifications, the Committee requires chemical characterization of the product in commerce along with data to be able to complete specifications related to the use of yeast extracts containing mannoproteins in wine manufacture. The following information is required:

- Composition of yeast extracts containing mannoproteins as well as the processes used in their manufacture;
- Analytical data from five batches of each commercial product, including information related to impurities; and
- Data on concentrations of yeast mannoproteins in wine in which yeast extracts containing mannoproteins have been used.

References

1. Bâgenholm V, Reddy SK, Bouraoui H, Morrill J, Kulcinskaja E, Bahr CM et al. (2017). Galactomannan catabolism conferred by a polysaccharide utilization locus of *Bacteroides ovatus*: Enzyme synergy and crystal structure of a β-mannanase. J Bio Chem. 6(292):229–43.
2. Bansal RA, Tadros S, Bansal AS (2017). Beer, cider and wine allergy. Case Reports Immunol. 2017:7958924.
3. den Besten G, Lange K, Havinga R, van Dijk TH, Gerding A, van Eunen K et al. (2013a). Gut-derived short-chain fatty acids are vividly assimilated into host carbohydrates and lipids. Am J Physiol Gastrointest Liver Physiol. 305(12):G900–10.
4. den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud D, Bakker BM. (2013b). The role of short-chain fatty acids in the interplay between diet, gut microbiota and host energy metabolism. J Lipid Res. 54(9):2325–40.
5. FAO/WHO (2016). 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 Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission (REP 16/FA).
6. FSANZ (2008). Yeast mannoproteins as a food additive for wine. Final assessment report. Application A605. Canberra: Food Standard Australia New Zealand.

7. González Pereyra ML, Dogi C, Torres Lisa A, Wittouck P, Ortíz M Escobar F et al. (2014). Genotoxicity and cytotoxicity evaluation of probiotic *Saccharomyces cerevisiae* RC016: A 60-day subchronic oral toxicity study in rats. J Appl Microbiol. 117:824–33.

8. Richeux F (2002). Assessment of sensitising properties on albino guinea pig maximisation test according to Magnusson and Kligman. Phycher Bio Développement report n°SMK-PH-02/0051.

9. Schauss AG, Glavits R, Endres J, Jensen GS, Clewell A (2012). Safety evaluation of a proprietary food-grade, dried fermentate preparation of *Saccharomyces cerevisiae*. Int J Toxicol. 31(1):36–45.

10. Tizard IR, Carpenter RH, McAnalley BH, Kemp MC (1989). The biological activities of mannans and related complex carbohydrates. Mol Biother. 1(6):290–6.

3.2 Revision of specifications and analytical methods

3.2.1 Microcrystalline cellulose

Microcrystalline cellulose was on the agenda of the present meeting for the revision of specifications related to its solubility in sodium hydroxide solution.

The Committee assessed the information submitted on the solubility of microcrystalline cellulose and redesignated its solubility as "Insoluble in water and ethanol. Practically insoluble or insoluble in sodium hydroxide solution (50 g/L)". The specifications were revised accordingly.

3.2.2 Silicon dioxide, amorphous

Silicon dioxide, amorphous was on the agenda at the present meeting for revisions related to pH, assay, loss on drying, loss on ignition and impurities. The Committee at its seventy-seventh meeting (Annex 1, reference *214*) evaluated silicon dioxide, amorphous as an anticaking agent. At its eightieth meeting (Annex 1, reference *223*), the Committee made the specifications tentative pending the receipt of further data and information.

The Committee at its present meeting received the requested information. The specifications were revised, and the tentative status was removed.

3.2.3 Sodium aluminium silicate

Sodium aluminium silicate was on the agenda at the present meeting for the revision of specifications. The Committee, at its eightieth meeting (Annex 1, reference *223*), made the specifications tentative and requested data on the solubility, the impurities soluble in 0.5 mol/L hydrochloric acid, and the suitability of the proposed assay method for the determination of aluminium, silicon and sodium. Information pertaining to functional uses other than anticaking agent was also requested.

At the current meeting, the Committee evaluated the data submitted for loss on ignition, impurities soluble in 0.5 mol/L hydrochloric acid and the assay.

Information received on functional uses confirmed that the substance is used only as an anticaking agent.

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

3.2.4 Steviol glycosides

Steviol glycosides was on the agenda at the present meeting for the revision of the method of assay. The Committee at its eighty-second meeting (Annex 1, reference *230*) made the specifications for steviol glycosides tentative pending receipt of a validated method capable of assaying additional steviol glycosides, as well as supporting validation data and information from five sample batches of steviol glycosides using the proposed method. The Committee received a validated HPLC–ultraviolet (UV) method for the assay of steviol glycosides, for which reference standards are commercially available. The presence of steviol glycosides that exist in small quantities is confirmed using an HPLC–mass spectrometric method and quantified using HPLC–UV data. The Committee also received assay data for three batches of a commercial product using the proposed methods. The Committee, at its present meeting, assessed the information received and replaced the existing assay. Two additional saccharides (galactose and arabinose) have been identified in the extracts of *Stevia rebaudiana* Bertoni since the last evaluation of steviol glycosides (Annex 1, reference *230*). The Committee included the two saccharides in the definition of the specifications for steviol glycosides from *Stevia rebaudiana* Bertoni.

The Committee received additional information pertaining to enzymatically modified steviol glycosides and further comments on the ADI established by the Committee at the sixty-ninth meeting (Annex 1, reference *190*). However, the Committee noted that the data were outside the scope of the call for the current meeting and, in the interest of transparency, did not consider them at the current meeting.

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

3.2.5 Sucrose esters of fatty acids

At the request of the Forty-eighth Session of the CCFA (FAO/WHO, 2016), sucrose esters of fatty acids was on the agenda of the present meeting for the revision of specifications related to solubility and to the chromatographic conditions in the assay method.

The Committee assessed the information submitted on the solubility of sucrose esters of fatty acids and revised the solubility criterion. In addition, the Committee reviewed the information submitted on the chromatographic conditions for the separation of the compounds and revised the UV integration instructions.

The specifications were revised.

References

1. FAO/WHO (2016). 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 Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission (REP 16/FA).

4. Future work and recommendations

General considerations

Information requirements for submissions on products derived from natural sources

The Committee emphasized that a full characterization of the products in commerce and a relevant set of biochemical and toxicological data on such products are essential in order to develop a specifications monograph and the related safety assessment.

Specific food additives

β-Carotenes

The Committee recommends that the group ADI for the sum of carotenoids, including β-carotene, β-apo-8′-carotenal and β-apo-8′-carotenoic acid methyl and ethyl esters, be re-evaluated in light of evidence that shows very low absorption of β-carotene in rodents and rabbits in contrast to humans.

Jagua (Genipin–Glycine) Blue

In order to complete an evaluation of the safety of Jagua (Genipin–Glycine) Blue, the Committee recommends that additional biochemical and toxicological information, including using higher doses of the "blue polymer" and including the dimers be submitted with the following:

- Characterization of the low molecular weight components of the "blue polymer";
- A validated method for the determination of dimers; and
- Data on concentrations of dimers from five batches of the commercial product.

Metatartaric acid

In order to remove the tentative designation from the specifications, the Committee recommends that the following information on the products of commerce be submitted by December 2018:

- Characterization of the products (optical rotation, content of free tartaric acid, degree of esterification and molecular weight distribution) and the corresponding analytical methods;
- Infrared spectrum (in a suitable medium); and

- Analytical results including the above parameters from a minimum of five batches of products currently available in commerce, along with quality control data.

Tannins

In order to complete specifications for oenological tannins used as an antioxidant, colour retention agent and stabilizer in wine, the Committee recommends that the following information be submitted for evaluation:

- Composition of tannins derived from the full range of raw materials as well as the processes used in their manufacture;
- Validated analytical method(s) and relevant quality control data;
- Analytical data from five batches of each commercial product including information related to impurities such as gums, resinous substances, residual solvents, sulfur dioxide content and metallic impurities (arsenic, lead, iron, cadmium and mercury);
- Solubility of the products in commerce, according to JECFA terminology; and
- Use levels, natural occurrence and food products in which tannins are used.

Submitters are encouraged to offer a rationale for a single specifications monograph for oenological tannins covering all products or individual monographs.

Yeast extracts containing mannoproteins

In order to remove the tentative designation of the specifications, the Committee recommends that the following information be submitted for evaluation:

- Composition of yeast extracts containing mannoproteins as well as the processes used in their manufacture;
- Analytical data from five batches of each commercial product, including information related to impurities; and
- Data on concentrations of yeast mannoproteins in wine in which yeast extracts containing mannoproteins have been used.

Acknowledgements

The Committee wishes to acknowledge that Ms Marla Sheffer, Ottawa, Canada, has provided outstanding editorial assistance, as WHO editor and co-rapporteur, in the preparation of this and numerous other reports and monographs over a period of 12 years. As Ms Sheffer has decided to retire, the Committee wishes to thank her for her dedication and excellent contribution. We wish her well in all of her future endeavours.

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-fourth meeting of JECFA.

References

1. Joint FAO/WHO Conference on Food Additives. Rome: Food and Agriculture Organization of the United Nations; 1956 (FAO Nutrition Meetings Report Series, No. 11); and Geneva: World Health Organization; 1956 (WHO Technical Report Series, No. 107).

2. FAO/WHO. Report of the Forty-ninth Session of the Codex Committee on Food Additives, Macau 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. Codex General Standard for Food Additives. Rome: Food and Agriculture Organization of the United Nations; and Geneva: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2015 (CODEX STAN 192-1995).

4. 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; 2009 (Environmental Health Criteria, No. 240; http://www.who.int/foodsafety/publications/chemical-food/en/, accessed 6 June 2017).

Annex 1

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

230. Evaluation of certain food additives (Eighty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 1000, 2016.

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

232. Safety evaluation of certain food additives. WHO Food Additives Series, No. 73, 2017.

233. Evaluation of certain contaminants in food (Eighty-third report of the Joint FAO/WHO Expert Committee on Food Additives) WHO Technical Report Series, No.1002, 2017.

ANNEX 2

Toxicological information, dietary exposures 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
Brilliant Blue FCF	R[a]	The Committee concluded that the available data support the revision of the ADI for Brilliant Blue FCF. In a long-term toxicity study in rats, a no-observed-adverse-effect level (NOAEL) of 631 mg/kg body weight (bw) per day was identified, based on a 15% decrease in mean terminal body weight and decreased survival of females at 1318 mg/kg bw per day. **The Committee established an ADI of 0–6 mg/kg bw** based on this NOAEL by applying an uncertainty factor of 100 for interspecies and intraspecies differences. The Committee noted that the conservative dietary exposure estimate of 5 mg/kg bw per day (95th percentile for children) is less than the upper limit of the ADI of 0–6 mg/kg bw established for Brilliant Blue FCF and **concluded that dietary exposure to Brilliant Blue FCF for children and all other age groups does not present a health concern.** **The previous ADI of 0–12.5 mg/kg bw was withdrawn.**
β-Carotene-rich extract from *Dunaliella salina*	N	The Committee noted that data have become available since the previous evaluation that show large differences in absorption of β-carotene between rodents and humans. The Committee considered that rodents are inappropriate animal models for establishing an ADI for β-carotene. The Committee noted that the toxicity of the other components of the β-carotene-rich d-limonene extract of *D. salina* (hereafter referred to as *D. salina* d-limonene extract) can be evaluated using the results of rodent studies. A short-term toxicity study in rats gave a NOAEL of 3180 mg/kg bw per day, the highest dose tested. No long-term toxicity or reproductive studies have been conducted. The *D. salina* d-limonene extract did not show genotoxicity or developmental toxicity. Correction of the NOAEL of 3180 mg/kg bw per day for the percentage of the algal component (20–35%) gives an adjusted NOAEL of 636–1113 mg/kg bw per day for the algal lipid component of the *D. salina* d-limonene extract. The margin of exposure for this algal lipid component is 2120–3710 using a dietary exposure of 18 mg/day (0.3 mg/kg bw per day). The Committee concluded that exposure to the algal component of the extract does not pose a health concern. The Committee noted that the total dietary exposure to β-carotene is not expected to increase when *D. salina* d-limonene extract is used as a food colour.

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions
		The Committee concluded that there was no health concern for the use of β-carotene-rich extract from *D. salina* when used as a food colour in accordance with the specifications established at this meeting. The Committee emphasized that this conclusion applies to the use of this extract as a food colour, not as a food supplement.
Fast Green FCF	R[a]	The ADI of 0–25 mg/kg bw established previously by the Committee was based on a long-term rat dietary that identified a NOAEL of 5% Fast Green FCF (equivalent to 2500 mg/kg bw per day), the highest concentration tested. The Committee concluded that the new data that had become available since the previous evaluation gave no reason to revise the ADI and **confirmed the ADI of 0–25 mg/kg bw.** The Committee noted that the conservative dietary exposure estimate for Fast Green FCF of 12 mg/kg bw per day (95th percentile for adolescents) was below the upper bound of the ADI. **The Committee concluded that dietary exposures to Fast Green FCF for adolescents and all other age groups do not present a health concern.**
Gum ghatti	R[b]	The Committee took into account the lack of systemic exposure to gum ghatti because of its high molecular weight and polysaccharide structure, its lack of toxicity in short-term studies, the lack of concern for genotoxicity and the absence of treatment-related adverse effects in studies of gum arabic and other polysaccharide gums with a similar profile. The Committee concluded that gum ghatti is unlikely to be of health concern and **established an ADI "not specified"[c] for gum ghatti that complies with the specifications.** **The Committee concluded that the estimated dietary exposure to gum ghatti of 12 mg/kg bw per day does not present a health concern.**
Jagua (Genipin–Glycine) Blue	N,T	The Committee noted that the highest doses tested in two 90-day toxicity studies in rats and dogs were only 330 and 338 mg/kg bw per day (expressed on a "blue polymer" basis[d]), respectively. The Committee was concerned that the possible effects of the low molecular weight component of the "blue polymer" that could be absorbed were not adequately investigated. A comparison of the dietary exposure estimate (11 mg/kg bw per day) with the NOAEL from the 90-day studies of oral toxicity in rats and dogs (approximately 330 mg/kg bw per day) gives a margin of exposure of about 30. Because of the limited biochemical and toxicological database and the low margin of exposure, **the Committee was unable to complete the evaluation for Jagua (Genipin–Glycine) Blue.**

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions
Metatartaric acid	T	As metatartaric acid undergoes enzymatic hydrolysis to tartaric acid prior to systemic absorption, the biochemical and toxicological data on tartaric acid considered at previous meetings are relevant to the safety assessment of metatartaric acid. Previously evaluated and new studies suggest no change to the group ADI previously established for L(+)-tartaric acid and its sodium, potassium and potassium–sodium salts, expressed as L(+)-tartaric acid. **The Committee concluded that metatartaric acid (when used in winemaking) should be included in the group ADI of 0–30 mg/kg bw for L(+)-tartaric acid and its sodium, potassium and potassium–sodium salts, expressed as L(+)-tartaric acid.** The Committee noted that the dietary exposure estimate for metatartaric acid for adult consumers of wine was 4% of the upper bound of the ADI **and concluded that dietary exposure to metatartaric acid in wine at the maximum use level of 100 mg/L does not present a health concern.**
Tamarind seed polysaccharide	N	The Committee noted the absence of toxicity in long-term rodent studies and lack of concern regarding genotoxicity, reproductive toxicity and developmental toxicity, and **established an ADI "not specified"[c] for tamarind seed polysaccharide.** **The Committee concluded that the estimated dietary exposure of 75 mg/kg bw per day based on proposed uses and use levels does not present a health concern.**
Tannins (oenological tannins)	–	The Committee noted that the available data do not provide clear information on which tannin sources and individual tannin compounds are present in commercially used oenological tannins and, thus, how the oenological tannins would compare to the tannins used in the submitted studies. Therefore, it is not possible to establish which studies are relevant and, consequently, the extent of the data gaps. The information on biochemical aspects is incomplete, with the implications of repeated dosing on absorption, tissue distribution and interindividual variation needing consideration. In general, there are also few data available on reproductive and developmental toxicity and/or long-term toxicity for some or all of the tannins. **In the absence of specifications and identification of the products in commerce, the Committee concluded that it was not possible to evaluate tannins used in winemaking.**
Yeast extracts containing mannoproteins	N,T	In addition to the natural presence of yeast mannoproteins in wine and the long history of consumption of yeast products in common foods, the Committee considered that the tentative product specifications for yeast extracts containing mannoproteins indicate that these do not contain chemical residues or microbiological contaminants of concern. In addition, the Committee estimated that dietary exposure to yeast mannoproteins due to the addition of yeast extracts containing mannoproteins to wine at the maximum level of 400 mg/L would result, on average, in a **20% increase in dietary exposure compared to the background exposure through the regular diet of 0.4–21 mg/kg bw per**

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions
		day, primarily driven by bread and pastries. These conservative dietary exposure estimates are based on the assumption that 100% of the yeast extracts containing mannoproteins is mannoproteins. In considering the data and information regarding yeast and yeast-derived products, **the Committee concluded that it is unlikely that there would be a health concern for the use of yeast extracts containing mannoproteins as a food additive for oenological uses at maximum use levels up to 400 mg/L for the stabilization of wine.** The Committee noted that any change in the uses and/or use levels of yeast extracts containing mannoproteins as a food additive will require a new evaluation.

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

[a] A maximum limit for manganese was added. High-performance liquid chromatography (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 or aqueous ammonium acetate.

[b] An HPLC method for the identification of the gum constituents was added to replace the thin-layer chromatography (TLC) method. One identity method, using a mercury-containing reagent, was removed. L-Rhamnose was added as one of the constituents of gum ghatti, based on current literature reports.

[c] ADI "not specified" is used to refer to a food substance of very low toxicity that, on the basis of the available data (chemical, biochemical, toxicological and other) and the total dietary exposure to the substance arising from its use at the levels necessary to achieve the desired effects and from its acceptable background levels in food, does not, in the opinion of the Committee, represent a hazard to health. For that reason, and for the reasons stated in the individual evaluations, the establishment of an ADI expressed in numerical form is not deemed necessary. An additive meeting this criterion must be used within the bounds of good manufacturing practice – i.e. it should be technologically efficacious and should be used at the lowest level necessary to achieve this effect; it should not conceal food of inferior quality or adulterated food; and it should not create a nutritional imbalance.

[d] "Blue polymer" refers to the blue-coloured genipin–glycine polymer and dimer content of Jagua (Genipin–Glycine) Blue.

Food additives considered for specifications only

Food additive	Specifications
Microcrystalline cellulose	R[a]
Silicon dioxide, amorphous	R[b]
Sodium aluminium silicate	R[c]
Steviol glycosides	R[d]
Sucrose esters of fatty acids	R[e]

R: existing specifications revised

[a] The Committee assessed the information submitted on the solubility of microcrystalline cellulose and redesignated its solubility as "Insoluble in water and ethanol. Practically insoluble or insoluble in sodium hydroxide solution (50 g/L)".

[b] Silicon dioxide, amorphous was on the agenda at the present meeting for revisions related to pH, assay, loss on drying, loss on ignition and impurities. The Committee at its present meeting received the requested information. The tentative status was removed.

[c] At the current meeting, the Committee evaluated the data submitted for loss on ignition, impurities soluble in 0.5 mol/L hydrochloric acid and the suitability of the proposed assay method for the determination of aluminium, silicon and sodium. Information received on functional uses confirmed that the substance is used only as an anticaking agent. The tentative status was removed.

[d] The Committee received a validated HPLC–ultraviolet (UV) method for the assay of steviol glycosides, for which reference standards are commercially available. The presence of steviol glycosides that exist in small quantities is confirmed using an HPLC–mass spectrometric method and quantified using HPLC–UV data. The Committee also received assay data for three batches of a commercial product using the proposed methods. The Committee, at its present meeting, assessed the information received and replaced the existing assay. Two additional saccharides (galactose and arabinose) have been identified in the extracts of *Stevia rebaudiana* Bertoni since the last evaluation of steviol glycosides. The Committee included the two saccharides in the definition of the specifications for steviol glycosides from *S. rebaudiana* Bertoni. The tentative status was removed.

[e] The Committee assessed the information submitted on the solubility of sucrose esters of fatty acids and revised the solubility criterion. In addition, the Committee reviewed the information submitted on the chromatographic conditions for the separation of the compounds and revised the UV integration instructions.

Annex 3

Meeting agenda

Food and Agriculture Organization of the United Nations

World Health Organization

84th JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES (JECFA)
FAO Headquarters, Rome, 6—15 June 2017

Opening:
Philippine Room (C277) 6 June at 9.30 h

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. C6. Critical issues and questions from Working Papers (first brief round of discussion on all subjects to inform the full Committee)

7. Evaluations

    7.1 Brilliant Blue FCF
    7.2 Carotenes from *Dunaliella salina*
    7.3 Fast Green FCF
    7.4 Gum ghatti
    7.5 Jagua (*Genipa americana*) extract
    7.6 Metatartaric acid
    7.7 Tamarind seed polysaccharide
    7.8 Tannins (oenological tannins)
    7.9 Yeast mannoproteins

8. Specifications

   8.1 Microcrystalline cellulose
   8.2 Silicon dioxide, amorphous
   8.3 Sodium aluminium silicate
   8.4 Steviol glycosides
   8.5 Sucrose esters of fatty acids

9. Other matters to be considered (general considerations)
   - Update from IPCS on risk assessment work: chemical-specific adjustment factors (CSAF), mixtures
   - Update of EHC240: (for information)
   - Development on guidance on the evaluation of genotoxicity studies
   - Updated guidance on dose–response modelling for the use in risk assessment
   - Exposure assessments

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 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)

Evaluation of Certain Food Additives
Seventy-ninth Report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 990, 2015 (124 pages)

Safety Evaluation of Certain Food Additives
Seventy-ninth Meeting of the Joint FAO/WHO Expert Committee on Food Additives
WHO Food Additives Series, No. 70, 2015 (369 pages)

Evaluation of Certain Veterinary Drug Residues in Food
Seventy-eighth Report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 988, 2014 (127 pages)

Toxicological Evaluation of Certain Veterinary Drug Residues in Food
Seventy-eighth Meeting of the Joint FAO/WHO Expert Committee on Food Additives
WHO Food Additives Series, No. 69, 2014 (241 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 and to prepare specifications for the identity and purity of the food additives.

The first part of the report contains a general discussion of the principles governing the toxicological evaluation of compounds on the agenda and includes information requirements for submissions on products derived from natural sources. Also described are updates on activities relevant to JECFA from the Forty-ninth Session of the Codex Committee on Food Additives (CCFA), the International Programme on Chemical Safety (IPCS) and JECFA publications. Next is a summary of the Committee's evaluations of technical, toxicological and dietary exposure data for nine food additives: Brilliant Blue FCF; β-carotene-rich extract from *Dunaliella salina*; Fast Green FCF; gum ghatti; Jagua (Genipin–Glycine) Blue; metatartaric acid; tamarind seed polysaccharide; tannins; and yeast extracts containing mannoproteins.

Specifications for the following food additives were revised: microcrystalline cellulose; silicon dioxide, amorphous; sodium aluminium silicate; steviol glycosides; and sucrose esters of fatty acids.

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 121016 4

SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 14 November 2019

doi: 10.2903/j.efsa.2019.5922

Opinion on the re-evaluation of acacia gum (E 414) as a food additive in foods for infants below 16 weeks of age and the follow-up of its re-evaluation as a food additive for uses in foods for all population groups

EFSA Panel on Food Additives and Flavourings (FAF),
Maged Younes, Gabriele Aquilina, Laurence Castle, Karl-Heinz Engel, Paul Fowler, Maria Jose Frutos Fernandez, Peter Fürst, Rainer Gürtler, Trine Husøy, Wim Mennes, Peter Moldeus, Agneta Oskarsson, Romina Shah, Ine Waalkens-Berendsen, Detlef Wölfle, Birgit Dusemund, Alicja Mortensen, Dominique Turck, Stefania Barmaz, Camilla Smeraldi, Alexandra Tard and Ursula Gundert-Remy

Abstract

EFSA is re-evaluating the safety of food additives already permitted in the Union before 20 January 2009 and issuing scientific opinions on their safety in line with Regulation (EC) No 1333/2008. Acacia gum (E 414) was re-evaluated in 2017 by the former EFSA Panel on Food Additives and Nutrient sources added to Food (ANS). As follow-up to this assessment, the Panel on Food Additives and Flavourings (FAF) was requested to assess the safety of acacia gum (E 414) as carry-over in food for infants below 16 weeks of age belonging to food categories 13.1.1 (Infant formulae) and 13.1.5.1 (Dietary foods for infants for special medical purposes and special formulae for infants) and to address the issues already identified during the re-evaluation of the food additive when used in food for the general population. The process involved the publication of a call for data to allow the interested parties to provide the requested information to complete the risk assessment. Based on the analytical data submitted in response to this call, the Panel recommended to lower the limits in the specifications for toxic elements and identified the need for further specifications for aluminium, microbiological criteria and protein residues. The Panel noted that information was not provided for oxidising enzymes and recommended that oxidases and peroxidases should be inactivated during the manufacturing process. The interested parties did not submit toxicological, clinical and post-marketing surveillance data specific for the assessment of the safety of acacia gum (E 414) in infants below 16 weeks of age. However, taking the highest doses tested without adverse effects from the subchronic studies available from the previous re-evaluation and comparing them with the estimated exposure in infants, the margins of safety were large indicating that there is no reason for health concern.

Keywords: Acacia gum, E 414, food additive, infants

Requestor: European Commission

Question Number: EFSA-Q-2018-00525

Correspondence: fip@efsa.europa.eu

Panel members: Maged Younes, Gabriele Aquilina, Laurence Castle, Karl-Heinz Engel, Paul Fowler, Maria Jose Frutos Fernandez, Peter Fürst, Rainer Gürtler, Ursula Gundert-Remy, Trine Husøy, Wim Mennes, Peter Moldeus, Agneta Oskarsson, Romina Shah, Ine Waalkens-Berendsen and Detlef Wölfle.

Suggested citation: EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), Younes M, Aquilina G, Castle L, Engel K-H, Fowler P, Frutos Fernandez MJ, Fürst P, Gürtler R, Husøy T, Mennes W, Moldeus P, Oskarsson A, Shah R, Waalkens-Berendsen I, Wölfle D, Dusemund B, Mortensen A, Turck D, Barmaz S, Smeraldi C, Tard A and Gundert-Remy U, 2019. Scientific Opinion on the re-evaluation of acacia gum (E 414) as a food additive in foods for infants below 16 weeks of age and the follow-up of its re-evaluation as a food additive for uses in foods for all population groups. EFSA Journal 2019;17 (12):5922, 23 pp. https://doi.org/10.2903/j.efsa.2019.5922

ISSN: 1831-4732

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

Summary

In accordance with Regulation (EU) No 257/2010, the European Food Safety Authority (EFSA) is currently re-evaluating the safety of food additives already permitted in the Union before 20 January 2009 and issuing scientific opinions on their safety when used in food as per Annexes II and III to Regulation (EC) No 1333/2008. The risk assessment approach followed in the re-evaluation has not covered the use of food additives in food for infants below 12 weeks of age. Additionally, while re-evaluating the safety of food additives referred to above, EFSA identified some concerns, namely (1) data gaps that have triggered recommendations in the published scientific opinions; and/or; (2) data gaps that have increased uncertainties linked to the risk assessment and/or which prevented the Panel from concluding on some aspects of it.

On 31 May 2017, EFSA published a guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age, thus enabling EFSA to assess the safety of food additive used in food for infants below this age. The age up to 16 weeks was selected in the guidance because infants are exposed to formula feeding until this age as the only source of food since complementary feeding is not supposed to be introduced before.

As follow-up, this Opinion addresses the data gaps previously identified during the re-evaluation of acacia gum (E 414) as a food additive in 2017 by the former EFSA Panel on Food Additives and Nutrient sources added to Food (ANS) and the safety in the special subpopulation of infants below 16 weeks of age.

The process followed involved the publication of a dedicated call for data allowing all interested parties to provide the requested information for completing the assessment and to confirm that the additive is present as carry-over in food categories 13.1.1 (Infant formulae) and 13.1.5.1 (Dietary foods for infants for special medical purposes and special formulae for infants). The data submitted in response to the call for data on acacia gum (E 414) comprised technical information and literature studies i.e. clinical studies on gastrointestinal effects in adults.

Acacia gum (E 414) is a dried exudate obtained from the stems and branches of natural strains of *Acacia senegal* (L.) Willdenow or closely related species. Specifications for acacia gum (E 414) have been defined in Commission Regulation (EU) No 231/2012. According to the submitter, the limits defined in the EU specification reflect the lowest technologically achievable levels for lead, mercury, cadmium and arsenic. The Panel, however, noted that the submitted data by the interested parties allow to lower the limits in the specifications for toxic elements and also identified the need for further specifications for aluminium, microbiological criteria and protein residues. The Panel further noted that no information was provided for oxidising enzymes. The Panel recommended that during the manufacturing process the oxidases and peroxidases present in acacia gum should be inactivated by heating to prevent the possible oxidative degradation of components in preparations to which acacia gum is added.

Information on particular specification requirements for identity and the purity of acacia gum (E 414) to be used in the food categories FC 13.1.1 and 13.1.5.1 (e.g. content residual proteins and enzymes, toxic elements) have been requested but were not provided. Analytical data on impurities in the final foods for infants below 16 weeks of age, when no legal limit has been established, were requested but were not provided. Analytical data on toxic elements in final infant formulae were not provided by the interested party. Therefore, the Panel considered that the manufacturers have no particular specifications requirements for the additive for the use in infant formulae.

According to Regulation (EC) No 1333/2008 (Annex III, part 5, section B), acacia gum (E 414) is authorised for use as a food additive in nutrient preparations intended to be used in foodstuffs for infants and young children, including food for infants below 16 weeks of age. Dietary exposure to acacia gum (E 414) from its use as a food additive was assessed based on maximum permitted level (MPL) set out in the EU legislation. The interested party confirmed that that the level of use of acacia gum (E 414) in infant formulae is compliant with this limit. The exposure scenario is based on the consumption levels recommended in the relevant Scientific Committee Guidance to be used in risk assessment; 200 and 260 mL formula/kg body weight (bw) per day as conservative mean and high level consumption values for 14- to 27-day-old infants. For infants below 16 weeks of age consuming infant formulae (FC 13.1.1) or infant food for special medical purpose (FSMP) (FC 13.1.5.1), mean exposure to acacia gum (E 414) was estimated to be 2 mg/kg bw per day while the high level was estimated at 2.6 mg/kg bw per day. The Panel also noted that the exposure estimates are based on the maximum levels for carry-over of acacia gum (E 414) from nutrient formulations used in infant formulae into the final product.

The interested party did not submit toxicological and clinical data which can be used to assess the safety of the acacia gum in infants below the age of 16 weeks. In addition, post-marketing surveillance data were not provided. However, in this special situation, where the exposure is low and only due to the carry over, a margin of safety (MOS) approach can be applied using available data from adult animals. Taking the highest doses tested without adverse effects in subchronic studies of 5,000 mg acacia gum/kg bw per day in rat and 20,000 mg acacia gum/kg bw per day in mice from the former EFSA evaluation in 2017 and comparing them with the exposure in infants of 2.6 mg/kg bw per day (high level estimate), MOS are roughly 2,000 and 8,000. These large MOS indicate that there is no reason for health concern. It is further noted that the data available from the former EFSA evaluation in 2017 did not show genotoxicity. Additionally, cases of allergenicity were not identified in the literature and in the former assessment.

Table of contents

1. Introduction

The present opinion deals with:

- the risk assessment of acacia gum (E 414) in food for infants below 16 weeks of age in the food categories (FC) 13.1.1 (Infant formulae as defined by Directive 2006/141/EC) and 13.1.5.1 (Dietary foods for infants for special medical purposes and special formulae for infants) according to uses in nutrient formulations authorised in section B of part 5 of Annex III to the Regulation (EC) No 1333/2008[1] on food additives.
- the follow-up on issues that have been expressed in the conclusions and recommendations of the Scientific Opinion on the re-evaluation of acacia gum (E 414) as a food additive (EFSA ANS Panel, 2017).

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

1.1.1. Background

The composition of food intended for infants and young children, as defined by Regulation (EU) No 609/2013[2], is regulated at EU level and such rules include requirements concerning the use of substances as food additives.

The use of food additives is regulated by Regulation (EC) No 1333/2008 on food additives. Only food additives that are included in the Union list, in particular in Annex II and III to that Regulation, may be placed on the market and used in food under the conditions of use specified therein.

In accordance with Regulation (EU) No 257/2010[3], EFSA is currently re-evaluating the safety of food additives already permitted in the Union before 20 January 2009 and issuing scientific opinions on their safety when used in food as per Annexes II and III to Regulation (EC) No 1333/2008. However, the risk assessment approach followed until now has not covered the use of food additives in food for infants below 12 weeks of age.

In addition, in these opinions EFSA identified some concerns, namely (1) Data gaps that have triggered recommendations in the published scientific opinions; and/or; (2) Data gaps that have increased uncertainties linked to the risk assessment and/or which prevented the Panel from concluding on some aspects of it.

On 31 May 2017, EFSA published a guidance document (EFSA Scientific Committee, 2017) on the risk assessment of substances present in food intended for infants below 16 weeks of age (SCF, 1998), thus enabling EFSA to assess the safety of food additive used in food for infants below 12 weeks of age. EFSA has launched dedicated calls for data to be able to perform such risk assessments.

The EC considers it is more effective that EFSA, in the context of these dedicated calls for data, also addresses all the issues and data gaps already identified in the relevant published scientific opinions on the re-evaluation of the safety of food additives permitted in food category 13.1.

In accordance with the current EC approach (European Commission, online) for the follow-up of EFSA's scientific opinions on the re-evaluation of the safety of permitted food additives for which some concerns have been identified, a specific call for data would be published by the EC on DG SANTE's website[4] on food additives and additional (missing) information would then be provided by interested parties to the EC.

However, for those scientific opinions on the re-evaluation of the safety of permitted food additives in food category 13.1 for which the risk assessment does not address their uses in food for infants below 12 weeks of age and for which some concerns have been identified by EFSA, the EC considers that for the sake of efficiency it would be appropriate to streamline the approach as described above.

[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 (EU) No 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control and repealing Council Directive 92/52/EEC, Commission Directives 96/8/EC, 1999/21/EC, 2006/125/EC and 2006/141/EC, Directive 2009/39/EC of the European Parliament and of the Council and Commission Regulations (EC) No 41/2009 and (EC) No 953/2009. OJ L 181, 29.6.2013, p. 35–56.

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

[4] https://ec.europa.eu/food/safety/food_improvement_agents/additives/re-evaluation_en

Therefore, the EC requests EFSA to address all the issues and data gaps already identified in the relevant published scientific opinions of those food additives (or groups of additives that can be addressed simultaneously) as part of the upcoming work on the safety assessment of food additives uses in food for infants below 12 weeks of age.

This follow-up aims at completing the re-evaluation of the food additives in question for all food categories, and includes calls for data covering the actual use and usage levels of food additives in food for both infants below 16[5] weeks of age as well as for older infants, young children and other groups of the population for which EFSA has already finalised its assessment.

The future evaluations of EFSA should systematically address the safety of use of food additives for all age groups, including the infants below 16 weeks of age.

1.1.2. Terms of Reference

In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002[6], and as part of EFSA's work in completing its risk assessments concerning the use of food additives in food for infants below 12 weeks of age,[5] covered by the re-evaluation programme and its terms of reference, the European Commission requests the European Food Safety Authority to address all the data gaps specified in the recommendations made in this scientific opinions on the re-evaluation of the safety of food additives permitted in food category 13.1 (food for infants and young children) of annex II to Regulation (EC) No 1333/2008.

1.1.3. Interpretation of Terms of Reference

The assessment will address the safety of acacia gum (E 414) in food intended for infants up to 16 weeks of age which are exposed to formula feeding until this age as the only source of food since complementary feeding is not supposed to be introduced before this age (see EFSA Scientific Committee, 2017).

1.2. Previous evaluations of acacia gum (E 414) for use in foods for infants

Acacia gum (E 414) was never formally evaluated by the EU Scientific Committee for Food (SCF). Nevertheless, acacia gum (E 414) was accepted for use in weaning food (SCF, 1991). In 1999, the SCF considered 'that the use of acacia gum/gum arabic in coatings for nutrient preparations containing trace elements is acceptable provided carry-over levels in infant formulae, follow-on formulae or FSMP[7] do not exceed 10 mg/kg' (SCF, 1999). In 1982 and 1990, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated acacia gum; based on the lack of adverse effects in the available toxicity studies, an acceptable daily intake (ADI) 'not specified' was allocated (JECFA, 1982, 1990).

Acacia gum has also been reviewed by the Nordic Council of Ministers (TemaNord, 2002), who concluded that even though the existing data do not raise any toxicological concern, allergy/ intolerance and the problem of marketing gums originating from acacia species not included in their evaluation should be considered in future evaluations.

1.3. Summary of the previous EFSA re-evaluation of acacia gum (E 414) for uses in food for all population groups except for infants below 12 weeks of age[8]

Under the frame of Regulation (EC) No 257/2010, the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) has re-evaluated the safety of acacia gum (E 414) when used as a food additive (EFSA ANS Panel, 2017).

[5] According to the EFSA Scientific Committee Guidance (EFSA Scientific Committee, 2017) this opinion will include infants up to 16 weeks of age.

[6] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.

[7] Food for special medical purposes.

[8] According to the EFSA Scientific Committee Guidance (EFSA Scientific Committee, 2017), this opinion will include infants up to 16 weeks of age.

In its scientific opinion, the ANS Panel reviewed available technical, biological and toxicological data on acacia gum (E 414) used as a food additive. Having considered the data available, the ANS Panel concluded that acacia gum is unlikely to be absorbed intact and is slightly fermented by the intestinal microbiota. The fermentation of acacia gum would lead to products considered of no safety concern by the ANS Panel such as short-chain fatty acids (SCFA). Acacia gum is considered to have low acute oral toxicity. Adverse effects were not reported in subchronic and carcinogenicity studies at the highest dose tested with the highest doses tested being 5,000 mg acacia gum/kg body weight (bw) per day in rat and 20,000 mg acacia gum/kg bw per day in mice and there was no concern with respect to genotoxicity. In the available studies addressing developmental and reproductive toxicity, effects were not reported up to the highest tested doses. Case reports on allergic reactions after oral exposure to acacia gum were not identified by the ANS Panel. The oral intake for up to 18 days of large amounts of acacia gum (up to 30,000 mg acacia gum/person per day approximately equivalent to 430 mg acacia gum/kg bw per day) was well tolerated in adults. Some individuals experienced flatulence which was considered by the ANS Panel as undesirable but not adverse effect. No cases of allergenicity after oral exposure to acacia gum were identified. Overall, the ANS Panel concluded that a numerical ADI was not needed for acacia gum (E 414) and that there was no safety concern for the general population at the refined exposure assessment (EFSA ANS Panel, 2017).

The ANS Panel, however, considered that the conclusions reached on the re-evaluation of the food additive were not applicable to the use of acacia gum (E 414) in food for infants under the age of 12 weeks.[5] The ANS Panel considered that these uses would require a specific risk assessment.

In addition, recommendations for revisions of the specifications were included in the ANS opinion. In particular, the ANS Panel noted that the detected levels of the toxic elements (lead, cadmium, mercury and arsenic) were far below those defined in the European Commission specifications for acacia gum, and therefore, recommended that the current limits should be lowered in order to ensure that acacia gum (E 414) as a food additive will not be a significant source of exposure to those toxic elements, in particular for infants and children. The ANS Panel also recommended the inclusion of limits for aluminium in the specifications. Furthermore, the inclusion of criteria for total aerobic microbial count (TAMC) and total combined yeast and mould count (TYMC) as well as limits for residual enzymatic activities and for protein content was recommended. Finally, the ANS Panel recommended that the oxidases and peroxidases in acacia gum should be inactivated during the manufacturing process to avoid any oxidative degradation of components in preparations to which acacia gum is added.

2. Data and methodologies

2.1. Data

EFSA launched a public call for data[9] and, if relevant, contacted other risk assessment bodies to collect relevant information from interested parties.

The Panel based its assessment on information submitted to EFSA following the public call for data, information from previous evaluations and additional available literature up to 2 October 2019.

The Mintel's Global New Products Database (GNPD) is an online database which monitors new introductions of packaged goods in the market worldwide. It contains information of over 3 million food and beverage products of which more than 1,100,000 are or have been available on the European food market. Mintel started covering EU's food markets in 1996, currently having 20 out of its 28 member countries and Norway presented in the Mintel's GNPD. This database was used to verify the use of the food additive acacia gum (E 414) in food products.

2.2. Methodologies

This opinion was formulated following the principles described in the EFSA Guidance on transparency with regard to scientific aspects of risk assessment (EFSA Scientific Committee, 2009) and following the relevant existing guidance documents from the EFSA Scientific Committee and in particular the EFSA Guidance of the Scientific Committee on the risk assessment of substances present in food intended for infants below 16 weeks of age (EFSA Scientific Committee, 2017).

[9] Call for technical and toxicological data on acacia gum (E 414) as a food additive for uses in foods for all population groups including infants below 16 weeks of age. Published: 10 October 2019. Available from: https://www.efsa.europa.eu/en/consultations/call/181010-4#_ftn2

In order to conclude on the safety of acacia gum (E 414), the FAF Panel assessed the information provided:

- for the follow-up on issues that have been raised in the conclusions and recommendations of the Scientific Opinion on the re-evaluation of acacia gum (E 414) as a food additive (EFSA ANS Panel, 2017); and
- for the risk assessment of acacia gum (E 414) in food for infants below 16 weeks of age in the FC 13.1.1 (Infant formulae as defined by Directive 2006/141/EC) and 13.1.5.1 (Dietary foods for special medical purposes and special formulae for infants) according to uses in nutrient formulations authorised in section B of part 5 of Annex III to the Regulation (EC) No 1333/2008[1] on food additives.

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

Dietary exposure to acacia gum (E 414) from its use as a food additive in nutrient formulations for use in foods for infants below 16 weeks of age was estimated combining the mean and highest consumption figures reported for the period of 14–27 days of life which corresponds to values of 200 and 260 mL/kg bw per day, respectively, with the maximum levels according to Annex III to Regulation (EC) No 1333/2008 and/or reported use levels and analytical data submitted to EFSA following a call for data. Different scenarios were used to calculate exposure (see Section 3.3.1). Uncertainties on the exposure assessment were identified and discussed.

3. Assessment

3.1. Technical data

3.1.1. Identity of the substance

According to Commission Regulation (EU) No 231/2012[10], the food additive 'E 414' is named as 'acacia gum'. A synonym for 'acacia gum' is 'gum arabic' (Commission Regulation (EU) No 231/2012[8]; JECFA, 2006). Acacia gum is a dried exudate obtained from the stems and branches of natural strains of *Acacia senegal* (L.) Willdenow or closely related species (Commission Regulation (EU) No 231/2012; JECFA, 2006).

3.1.2. Specifications

The specifications for acacia gum (E 414) as defined in the Commission Regulation (EU) No 231/2012 and as proposed by JECFA (2006) are listed in Table 1.

[10] Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council (Text with EEA relevance).

Table 1: Specifications for acacia gum (E 414) according to Commission Regulation (EU) No 231/2012 and proposed by JECFA (2006)

	Commission Regulation (EU) No 231/2012	**JECFA (2006)**
Definition	Acacia gum is a dried exudation obtained from the stems and branches of strains of *Acacia senegal* (L) Willdenow or closely related species of *Acacia* (family Leguminosae). It consists mainly of high molecular weight polysaccharides and their calcium, magnesium and potassium salts, which on hydrolysis yield arabinose, galactose, rhamnose and glucuronic acid	Gum arabic is a dried exudate obtained from the stems and branches of *Acacia senegal* (L.) Willdenow or *Acacia seyal* (fam. Leguminosae) Gum arabic consists mainly of high-molecular weight polysaccharides and their calcium, magnesium and potassium salts, which on hydrolysis yield arabinose, galactose, rhamnose and glucuronic acid. Items of commerce may contain extraneous materials such as sand and pieces of bark, which must be removed before use in food
Synonym	Gum arabic	Gum arabic (*Acacia senegal*), gum arabic (*Acacia seyal*), Acacia gum, arabic gum, INS No. 414
CAS Numbers		9000-01-5
EINECS	232-519-5	
Molecular weight	Approximately 350,000	
Description	Unground acacia gum occurs as white or yellowish-white spheroidal tears of varying sizes or as angular fragments and is sometimes mixed with darker fragments. It is also available in the form of white to yellowish-white flakes, granules, powder or spray-dried material	Gum arabic (*A. senegal*) is a pale white to orange-brown solid, which breaks with a glassy fracture. The best grades are in the form of whole, spheroidal tears of varying size with a matt surface texture. When ground, the pieces are paler and have a glassy appearanceGum arabic (*A. seyal*) is more brittle than the hard tears of gum arabic (*A. senegal*) Gum arabic is also available commercially in the form of white to yellowish white flakes, granules, powder, roller dried or spray-dried material An aqueous solution of 1 g in 2 mL flows readily and is acid to litmus
Identification		
Solubility	1 g dissolves in 2 mL of cold water forming a solution which flows readily and is acid to litmus; insoluble in ethanol	1 g dissolves in 2 mL of water; insoluble in ethanol
Gum constituents		Proceed as directed under Gum Constituents Identification (FNP 5) using the following as reference standards: arabinose, galactose, mannose, rhamnose, galacturonic acid, glucuronic acid and xylose. Arabinose, galactose, rhamnose and glucuronic acid should be present. Additional spots corresponding to mannose, xylose and galacturonic acid should be absent
Optical rotation		Gum from *A. senegal*: aqueous solutions are levorotatory Gum from *A. seyal*: aqueous solutions are dextrorotatory Test a solution of 10 g of sample (dry basis) in 100 mL of water (if necessary, previously filtered through a No. 42 paper or a 0.8 μm Millipore filter), using a 200-mm tube

	Commission Regulation (EU) No 231/2012	JECFA (2006)
Purity		
Loss on drying	Not more than 17% (105°C, 5 h) for granular and not more than 10% (105°C, 4 h) for spray-dried material	Not more than 15% (105°, 5 h) for granular and not more than 10% (105°, 4 h) for spray-dried material Unground samples should be powdered to pass through a No. 40 sieve and mixed well before weighing
Total ash	Not more than 4%	Not more than 4%
Acid insoluble ash	Not more than 0.5%	Not more than 0.5%
Acid insoluble matter	Not more than 1%	Not more than 1%
Starch or dextrin	Boil a 1 in 50 solution of the gum and cool. To 5 mL add 1 drop of iodine solution. No bluish or reddish colours are produced	Boil a 1 in 50 solution of the sample, cool and add a few drops of Iodine T.S. No bluish or reddish colour should be produced
Tannin	To 10 mL of a 1 in 50 solution, add about 0.1 mL of ferric chloride solution (9 g $FeCl_3 \cdot 6H_2O$ made up to 100 mL with water). No blackish coloration or blackish precipitate is formed	To 10 mL of a 1 in 50 solution of the sample, add about 0.1 mL of ferric chloride TS. No blackish colouration or blackish precipitate should be formed
Arsenic	Not more than 3 mg/kg	
Lead	Not more than 2 mg/kg	Not more than 2 mg/kg
Mercury	Not more than 1 mg/kg	
Cadmium	Not more than 1 mg/kg	
Hydrolysis products	Mannose, xylose and galacturonic acid are absent (determined by chromatography)	
Microbiological criteria		
Salmonella spp.	Absent in 10 g	Negative per test
Escherichia coli	Absent in 5 g	Negative in 1 g

CAS: Chemical Abstracts Service; EINECS: European Inventory of Existing Commercial Substances.

3.1.2.1. Analytical data from commercial samples of the food additive

Analytical data for acacia gum as raw and spray-dried material have been provided by one of the interested parties in response to the call for data (Documentation provided to EFSA n. 1). According to the submitter, the limits defined in Regulation (EC) No 231/2012 reflect the lowest technologically achievable levels for lead, mercury, cadmium and arsenic. The Panel noted that the analytical data on toxic elements submitted by the interested party also in response to the former call for data (EFSA ANS Panel, 2017) were substantially lower. In the current data submission from the interested party, all the data (n = 29) for arsenic, cadmium and mercury were < 0.05, < 0.01 and < 0.008 mg/kg, respectively. For lead, 29 sample results of raw and spray-dried material were provided in the current submission. The levels ranged between < 0.005 and 0.048 mg/kg (median: < 0.02 mg/kg, mean: 0.029 mg/kg, 90th percentile: 0.043 mg/kg).

A lowest technologically achievable level of 100–120 mg/kg is proposed for aluminium (not currently included in the specification) by the interested party. The Panel noted that in the former re-evaluation the analytical data for aluminium ranged between 3.71 and 14.74 mg/kg while in the current data submission (n = 29), the aluminium level ranged between 3.1 and 235.2 mg/kg (median: 25.9 mg/kg; 90th percentile: 99.5 mg/kg).

The interested party proposed that the lowest total aerobic microbial count (TAMC) should be 10,000 CFU/g and total combined yeast and mould count (TYMC) 1,000 CFU/g. The Panel noted that in 12 samples of raw material the TAMC ranged between 240 and 8,200 CFU/g and the TYMC between < 10 and 13,000 CFU/g. For 13 samples of spray-dried material, the corresponding values were < 10 to 7,400 CFU/g and < 10 and 90 CFU/g, respectively. The Panel further noted that the TYMC counts

are nearly exclusively due to moulds and the moulds count are substantially lower in the spray-dried material compared to the raw material.

Analytical data on current levels of residual proteins in the acacia gum (E 414) preparations were provided; the lowest technologically achievable level for residual protein was proposed to be set at not more than 3.5%. Analytical data on toxic elements in final infant formulae were not provided by the interested party. Information on the lowest technologically achievable levels for oxidising enzymes (oxidases and peroxidases) requested in the call for data were not provided, it should be clarified that enzymes are part of the protein fraction.

Ten commercial samples of spray-dried acacia gum (E 414) were tested for *Cronobacter (Enterobacter) sakazakii* (method ISO/TS 22964), five for Salmonella spp. (method NF EN ISO 6579-15 (A)) and *Listeria monocytogenes* (method NF EN ISO 11290-1(A)). All tested samples (between 25 and 250 g) were negative (Documentation provided to EFSA n. 1).

3.1.3. Proposed revision to existing EU Specifications for E 414

Based on the analytical data provided by the Association for International Promotion of Gums in response to the recommendations issued by the ANS Panel, the FAF Panel recommends the revisions listed in Table 2.

Table 2: Proposal for a revised version of the existing EU Specifications for acacia gum (E 414)

Commission Regulation (EU) No 231/2012		**Comment/Justification for revision**
Definition	Acacia gum is a dried exudation obtained from the stems and branches of strains of *Acacia senegal* (L) Willdenow or closely related species of *Acacia* (family Leguminosae). It consists mainly of high molecular weight polysaccharides and their calcium, magnesium and potassium salts, which on hydrolysis yield arabinose, galactose, rhamnose and glucuronic acid	Unchanged
Synonyms	Gum arabic	Unchanged
EINECS	232-519-5	Unchanged
Molecular weight	Approximately 350,000	Unchanged
Description	Unground acacia gum occurs as white or yellowish-white spheroidal tears of varying sizes or as angular fragments and is sometimes mixed with darker fragments. It is also available in the form of white to yellowish-white flakes, granules, powder or spray-dried material	Unchanged
Identification		
Solubility	1 g dissolves in 2 mL of cold water forming a solution which flows readily and is acid to litmus; insoluble in ethanol	Unchanged
Purity		
Loss on drying	Not more than 17 % (105 °C, 5 h) for granular and not more than 10 % (105 °C, 4 h) for spray-dried material	Unchanged
Total ash	Not more than 4 %	Unchanged
Acid insoluble ash	Not more than 0.5 %	Unchanged
Acid insoluble matter	Not more than 1 %	Unchanged
Starch or dextrin	Boil a 1 in 50 solution of the gum and cool. To 5 mL add 1 drop of iodine solution. No bluish or reddish colours are produced	Unchanged

Commission Regulation (EU) No 231/2012		Comment/Justification for revision
Tannin	To 10 mL of a 1 in 50 solution add about 0.1 mL of ferric chloride solution (9 g $FeCl_3 \cdot 6H_2O$ made up to 100 mL with water). No blackish coloration or blackish precipitate is formed	Unchanged
Arsenic		Current levels (3 mg/kg) should be lowered on the basis of the analytical data provided and taking into account the measurement uncertainty
Lead		Current levels (2 mg/kg) should be lowered on the basis of the analytical data provided and taking into account the measurement uncertainty
Mercury		Current levels (1 mg/kg) should be lowered on the basis of the analytical data provided and taking into account the measurement uncertainty
Cadmium		Current levels (1 mg/kg) should be lowered on the basis of the analytical data provided and taking into account the measurement uncertainty
Aluminium		Added. The levels should be defined on the basis of the analytical data provided and taking into account the measurement uncertainty
Hydrolysis products	Mannose, xylose and galacturonic acid are absent (determined by chromatography)	Unchanged
Proteins	< 3.5%	Added on the basis of the available data
Microbiological criteria		
Salmonella spp.	Absent in 250 g	Changed on the basis of the available data
Escherichia coli	Absent in 5 g	Unchanged
Cronobacter sakazakii	Negative in 150 g	Added on the basis of the available data*
Listeria monocytogenes	Negative in 125 g	Changed on the basis of the available data
TYMC	< 100/g	Added on the basis of the available data*
TAMC	< 10^4/g	Added on the basis of the available data*

EINECS: European Inventory of Existing Commercial Substances; TYMC: total combined yeast and mould count; TAMC: total anaerobic microbial count.
*: Based on data on the spray-dried acacia gum.

While interested parties stated that the current limits defined in Regulation (EC) No 231/2012 for lead, arsenic, cadmium and mercury reflect the lowest technologically achievable levels, the analytical data submitted show that their actual concentrations are substantially lower. The Panel considered that the maximum limits in the EU specifications for toxic elements should be established based on actual levels in the food additive. Therefore, if the European Commission decides to revise the current limits in the EU specifications to more realistic values, the following calculations could be considered.

Using the analytical data provided by the interested parties on the content of arsenic (As), cadmium (Cd), and mercury (Hg) in samples of acacia gum, which were all below the limit of detection (LOD) of 0.005, 0.01 and 0.008 mg/kg, respectively, and multiplying these by an 'uncertainty' factor of 10 to cover uncertainties, such as representativeness, homogeneity and analytical measurements, the maximum limit values for the revision of the EU specifications would be 0.5, 0.1 and 0.1 mg/kg, respectively. For

lead (Pb), some levels in acacia gum submitted by the interested parties were above the LOD. Thus, the P90 of 0.04 mg/kg may be used as a limit value for the revision of the EU specification for lead.

According to interested parties, the lowest technologically achievable level for aluminium (Al) is 100–120 mg/kg acacia gum. The Panel also considered that a maximum limit for aluminium should be included in the EU specifications. In the absence of a specific limit proposed from interested parties, using the analytical data provided and taking the P90 value, a maximum limit of 100 mg Al/kg may be included in the EU specifications.

The Panel emphasises that the choice of the 'uncertainty' factor and the percentile to conclude on the maximum limits for toxic elements in the specifications is in the remit of risk management.

In the earlier opinion on acacia gum (EFSA ANS Panel, 2017) the most exposed population group was toddlers. The Panel considered that the non-brand-loyal scenario covering the general population was the more appropriate and realistic scenario for risk characterisation. In that scenario, the highest P95 in toddlers was 719 mg/kg bw day. The above mentioned maximum limits for the toxic elements combined with the intake of acacia gum of (719 mg/kg bw per day) would result in an exposure which can be compared with the following health-based guidance values or reference values for the five toxic elements: a tolerable weekly intake (TWI) of 2.5 μg/kg bw for cadmium (EFSA CONTAM Panel, 2009), a TWI of 4 μg/kg bw for mercury (EFSA CONTAM Panel, 2012), a tolerable weekly intake (TWI) of 1,000 μg/kg bw for aluminium (EFSA, 2008), a BMDL01 of 0.5 μg/kg bw per day for lead (EFSA CONTAM Panel, 2010) and a BMDL01 of 0.3 to 8 μg/kg bw per day for arsenic (EFSA CONTAM Panel, 2009).

The outcome of such an exercise illustrates the health impact that would result if the specification values mentioned above were to be used: for Cd, Hg and Al the exhaustion of the health based guidance values would be 20%, 13% and 50%, respectively; for Pb and As the MOS/MOE would be 17 and 0.8–22, respectively. This supports the recommendation to decrease the current maximum limits for lead, arsenic, cadmium and mercury and to include a new limit to aluminium in the EU specification for acacia gum (E 414) considering also other sources of exposure.

3.1.3.1. Information on particular specification requirements for the additive for use in infant formulae

Information on particular specification requirements for identity and the purity of acacia gum (E 414) to be used in the food categories FC 13.1.1 and 13.1.5.1 (e.g. content residual proteins and enzymes, toxic elements) have been requested but were not provided. Analytical data on impurities in the final foods for infants below 16 weeks of age, when no legal limit has been established, were requested but were not provided. Analytical data on toxic elements in final infant formulae were not provided by the interested party. Therefore, the Panel considered that the manufacturers have no particular specifications requirements for the additive for the use in infant formulae.

3.1.3.2. Stability of the substance, and reaction and fate in food

No data were provided in response to the call for data, concerning the stability of the additive and possible reactions and fate in foods for infants below 16 weeks of age (i.e. infant formulae). There was the simple statement that; no reaction products are known to occur in this food category (Documentation provided to EFSA n. 1). The earlier evaluation of acacia gum (EFSA ANS Panel, 2017) observed that only limited information on reaction and fate of acacia gum in foods was available but noted that the gum is stable in acid conditions and also has excellent heat stability. So, it can be concluded that no undesirable reactions of the gum are to be expected if used in infant formulae.

Although not concerning reactions and fate of the gum itself, the ANS Panel in 2017 recommended that the oxidases and peroxidases in acacia gum should be inactivated during the manufacturing process to avoid any oxidative degradation of components in preparations to which acacia gum is added (EFSA ANS Panel, 2017). Any such oxidative reactions would also be of potential concern for use of the additive in infant formulae. As described in Section 3.1.2 above, the call for data did not elicit any information on the lowest technologically achievable levels for oxidases and peroxidases. This topic is considered further in the discussion and recommendations sections of the current Opinion.

3.2. Authorised uses and use levels

Maximum levels of acacia gum (E 414) in foods for infants below 16 weeks of age are defined in Regulation (EC) No 1333/2008 on food additives, as amended. In this opinion, these levels are termed maximum permitted levels (MPLs).

According to Regulation (EC) No 1333/2008 (Annex III, part 5, section B), acacia gum (E 414) is authorised as a food additive in nutrient preparations intended to be used in foodstuffs for infants and young children, including infants below 16 weeks of age. The MPLs in all nutrient preparations are set at 150,000 mg/kg and as carry-over at 10 mg/kg in the final products, including food categories 13.1.1 (Infant formulae as defined Directive 2006/141/EC) and 13.1.5.1 (Dietary foods for infants for special medical purposes and special formulae for infants).

Table 3: MPLs of acacia gum (E 414) in foods for infants below 16 weeks of age according to Annex III, Part 5, Section B to Regulation (EC) No 1333/2008

E number	Name of the food additive	Maximum permitted level	Nutrient to which the food additive may be added	Food category
E 414	Acacia gum	150,000 mg/kg in the nutrient preparation and 10 mg/kg carry-over in final products	All nutrients	Foods for infants and young children

3.3. Exposure data

Some food additives are authorised in the EU in infants' formulae as defined by Commission Directive 2006/141/EC (FC 13.1.1) and in dietary foods for infants for special medical purposes and special formulae for infants (FC 13.1.5.1) at a specific MPL. However, a food additive may be used at a lower level than the MPL. Therefore, actual use levels are required for performing a more realistic exposure assessment.

In the framework of Regulation (EC) No 1333/2008 on food additives and of Commission Regulation (EU) No 257/2010 regarding the re-evaluation of approved food additives, EFSA issued a public call[11] for technical and toxicological data on acacia gum (E 414) as a food additive for uses in foods for all population groups including infants below 16 weeks of age. In response to this public call, information on the actual use levels of acacia gum (E 414) in foods was made available to EFSA by industry. No analytical data on the concentration of acacia gum (E 414) in foods were made available by the Member States.

3.3.1. Reported use levels in food category 13.1.1 and 13.1.5.1 as a carry-over from the authorised use according to Annex III to Regulation No 1333/2008, Part 5, Section B

Industry did not provide EFSA with use levels but indicated that the MPL of 10 mg/kg final food set in Annex III to Regulation No 1333/2008, is the level used whenever a nutrient preparation containing acacia gum (E 414) is used in FC 13.1.1 or FC 13.1.5.1. Therefore, the assumption of 10 mg/kg final food is also the one taken on for the refined exposure assessment scenario.

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

The Mintel's GNPD is an online database which monitors new introductions of packaged goods in the market worldwide. It contains information of over 3 million food and beverage products of which more than 1,100,000 are or have been available on the European food market. Mintel started covering EU's food markets in 1996, currently having 25 out of its 28 member countries and Norway presented in the Mintel GNPD.[12]

For the purpose of this Scientific Opinion, the Mintel's GNPD[13] was used for checking the labelling of food and beverage products and food supplements for acacia gum (E 414) within the EU's food market as the database contains the compulsory ingredient information on the label.

[11] Call for technical and toxicological data on acacia gum (E 414) for uses as a food additive in foods for all population groups including infants below 16 weeks of age. Available from: http://www.efsa.europa.eu/sites/default/files/consultation/callsforda ta/2018-00775_call_for_data_acacia_gum_E_414.pdf

[12] Missing Cyprus, Luxembourg and Malta.

[13] http://www.gnpd.com/sinatra/home/ accessed on 16/5/2019.

No products intended for use in infants below 16 weeks were found in the Mintel's GNPD as labelled with acacia gum (E 414). The additive is only authorised according to Annex III for which the labelling is not mandatory.

3.4. Exposure estimates for infants below 16 weeks

Exposure to acacia gum (E 414) from its uses as a food additive in nutrient preparations intended to be used in formulae for infants below 16 weeks was estimated. This scenario is based on the - consumption levels recommended in the relevant SC Guidance (EFSA Scientific Committee, 2017) to be used in risk assessment. This guidance 'recommends values of 200 and 260 mL formula[14] /kg bw per day as conservative mean and high level consumption values to be used for performing the risk assessments of substances which do not accumulate in the body present in food intended for infants below 16 weeks of age'. These recommended consumption levels correspond to 14- to 27-day-old infants' consumption. For the regulatory maximum level exposure assessment scenario, the MPL for infant formulae (10 mg/kg for both FC 13.1.1 and FC 13.1.5.1) was used as well as for the refined scenario.

3.4.1. Dietary exposure to acacia gum (E 414) from infant formulae

Table 4 summarises the estimated exposure to acacia gum (E 414) from its use as a food additive in nutrient preparations added to both, FC 13.1.1 and FC 13.1.5.1 for infants below 16 weeks of age (Table 4).

Table 4: Dietary exposure to acacia gum (E 414) in foods for infants below 16 weeks of age according to Annex III, Part 5, Section B to Regulation (EC) No 1333/2008 (in mg/kg bw per day)

	Infants (< 16 weeks of age)
Regulatory maximum level exposure assessment scenario/Refined estimated exposure assessment scenario	
• Mean consumption (200 mL/kg bw per day)	2
• High level consumption (95th percentile, 260 mL/kg bw per day)	2.6

bw: body weight.

Only one scenario was estimated as regulatory and refined scenarios use the same level.

3.4.2. Uncertainty analysis

In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2007), the following sources of uncertainty have been considered and summarised in Table 5.

Table 5: Qualitative evaluation of influence of uncertainties on the dietary exposure estimate

Sources of uncertainties	**Direction[(a)]**
Consumption data: one reference point only to estimate exposure during the period of up to 16 weeks of age	+/–
Regulatory maximum level exposure assessment scenario: – exposure calculations based on the MPL according to Annex III to Regulation (EC) No 1333/2008 (carry-over)	+
Refined exposure assessment scenarios: – exposure calculations based on the maximum level	+

MPL: maximum permitted level.
(a): +, uncertainty with potential to cause overestimation of exposure; –, uncertainty with potential to cause underestimation of exposure.

Acacia gum (E 414) is authorised as a food additive in nutrient preparations used in foods for infants (FC 13.1) according to Annex III to Regulation (EC) No 1333/2008. Based on the assumption that carers of children would be brand-loyal to an infant formula (FC 13.1.1) or infant formulae for

[14] Editorial.

special medical purposes (FC 13.1.5.1), the exposure assessment scenario (Table 4) would in general result in a reliable estimation of exposure.

3.5. Biological and toxicological data

3.5.1. Previous evaluation by ANS Panel (2017)

The following text (in italics) is from the opinion published in 2017 (EFSA ANS Panel, 2017). New information and assessments related to the specific age group below 16 weeks of age are added in the following paragraph.

The in vitro degradation and the in vivo digestibility of acacia gum have been investigated in animals and humans models and in a human study. The ANS Panel considered that these data indicated that acacia gum would be not absorbed intact but fermented by enteric bacteria in humans. The rate of hydrolysis in the gastrointestinal tract in humans is unknown; however, the ANS Panel considered that acacia gum is unlikely to be absorbed intact, and that the limited extent of its fermentation would lead to products such as SCFA which were considered of no safety concern by the ANS Panel.

Acacia gum was regarded by the ANS Panel as having a low acute oral toxicity.

In a subacute toxicity study (Anderson et al.*, 1984), no histopathological changes were identified by electron microscopic examination of organs from rats fed diets containing 1–8% acacia gum daily (equivalent to 1,180–9,440 mg acacia gum/kg bw per day) for 28 days.*

Among other studies, the subchronic (13 weeks) oral toxicity of acacia gum was investigated by Anderson et al. *(1982). The animals received acacia gum in their diet and the study was conducted in two consecutive experimental phases. In the first one, the rats were given doses ranging from 0 to about 5,000 mg acacia gum/kg bw per day and in the second phase, they received 0 or 14,000 mg acacia gum/kg bw per day. The Panel noted that these two studies were done independently and that merging their data may not be straightforward. The ANS Panel considered that no toxicological effect was observed in these studies by Anderson* et al. *(1982). From the first study, no adverse effects have been identified up to 5,220 and 5,310 mg acacia gum/kg bw per day in male and female, respectively, the highest dose tested.*

Overall, the short-term and subchronic administration of oral doses up to 5,000 mg acacia gum/kg bw per day to rats and 20,000 mg acacia gum/kg bw per day to mice, the highest doses tested, did not induce any biologically relevant adverse effects. In some studies, caecal enlargement was observed. The ANS Panel considered that an increased caecum weight in animals fed high amounts of carbohydrates is considered as a physiological response to an increased fermentation by the intestinal microbiota.

Based on the data available, the ANS Panel considered that there was no concern with respect to the genotoxicity of acacia gum.

No chronic toxicity studies according to OECD guidelines (452) or equivalent were identified by the ANS Panel.

Acacia gum was tested for carcinogenicity in rats and mice receiving diets containing 2.5% and 5% acacia gum in the feed for 103 weeks equivalent to 1,250 and 2,500 mg acacia gum/kg bw per day in rats, and 3,750 and 7,500 mg acacia gum/kg bw per day in mice (NTP, 1982; Melnick et al.*, 1983). From this study, the ANS Panel considered that acacia gum is not of concern with respect to carcinogenicity.*

In a dietary combined fertility and developmental toxicity study in rats (Collins et al.*, 1987), a NOAEL of 10,647 mg acacia gum/kg bw per day for reproductive, developmental and parental effects was identified, the highest dose tested. In addition, other reproductive studies in rats showed no effects at the highest dose tested (Morseth and Ihara 1989a, Huynh* et al.*, 2000). In the identically performed prenatal developmental tests with acacia gum by gavage in mice, rats and hamsters (FDRL, 1972b), 1,600 mg/kg bw per day (the highest doses tested) showed no dose-related developmental effects.*

No case reports on allergic reaction after oral exposure to acacia gum could be identified by the Panel.

In humans, the repeated oral daily intake of a large amount of acacia gum up to 30 g (approx. 430 mg acacia gum/kg bw per day) for up to 18 days was well tolerated and had only a minimum effect on stool weight and decrease in serum cholesterol. Some individuals experienced flatulence which was considered by the Panel as undesirable but not adverse.

3.5.2. Newly available data

Toxicological data

No new toxicological data were submitted by the interested parties which allow to assess the safety of acacia gum (E 414) when used in foods for infants below 16 weeks of age.

The Panel, performing a literature search, identified a publication on the effects of gum arabic[15] in which development, behaviour and biochemical parameters were tested after administration via drinking water of 0, 1 and 4 mg/kg bw per day to female mice (Swiss-Webster strain) from gestation day (GD) 0 to postnatal day (PND) 15 (Binjumah et al., 2018).

When reviewing the publication, the Panel noted that the study and the reporting showed several serious flaws and, therefore, considered that the study cannot be used for risk assessment.

Clinical data

Clinical data from two studies in adults have been submitted focusing on gastrointestinal effects.

In the study of Bliss et al. (2001), patients with stool incontinence were treated with psyllium, gum arabic,[15] or a placebo for 31 days after a run-in period of 8 days. From 42 patients (age 34–76 years; 62 ± 3 (mean ± SEM) years) recruited for the study 39 completed the study according to the protocol. The dose of gum arabic was 25 g/day given mixed with half-strength fruit juice in two servings (morning and evening). Whereas the proportion of 'incontinent' stools was significantly less in the gum arabic group compared to the control, no influence on the frequency of flatus (in the initial period the dose was given in increasing doses until after 6 days the full dose was reached) and on SCFAs concentration in stool was observed.

In the double-blinded, controlled study of Calame et al. (2008) 54 healthy volunteers (age 30.6 ± 13 years) were randomly assigned to six groups being treated with water as control, or 5, 10, 20 and 40 g of EmulGold (which is a tradename of gum arabic[17]) per day or inulin as positive control (10 g Fibruline per day which is the tradename) over 4 weeks. The primary endpoint was the change in microbiota whereby the genera of *Bifidobacteria* and lactobacilli were taken as potentially beneficial bacteria and those of *Bacteroides*, *Clostridium difficile* and enterococci as potentially non-beneficial. The secondary endpoint was side effects, in particular diarrhoea. There were some statistically significant changes in the numbers of *Bifidobacteria* and lactobacilli (increase at 10 g/day) and also in *Bacteroides* (increase at 10 g/day). The Panel considered the changes as not clinically meaningful. Gastrointestinal side effects, in particular diarrhoea, did not occur more frequently in all treated groups compared to control.

In summary, the two studies in adults did not show adverse effects of gum arabic up to a dose of 40 g/day (0.64 g/kg bw per day, calculated with the actual weight) in healthy volunteers treated over 4 weeks and up to 25 g/day (0.30 g/kg bw per day, calculated with the actual weight) in patients with stool incontinence. However, one study focuses on effects on the microbiome which cannot be evaluated at the present state of knowledge and the other was performed in patients with stool incontinence. In addition, these studies in adults cannot be used to assess the safety of the use of acacia gum in infants below 16 weeks of age.

Post-marketing data

The interested parties did not submit post-marketing surveillance reports on undesired and adverse reactions requested in the call for data.

3.6. Discussion

According to the submitter, the limits defined in Regulation (EC) No 231/2012 reflect the lowest technologically achievable levels for lead, mercury, cadmium and arsenic. The Panel, however, noted that the submitted data by the interested parties allow to lower the limits in the specifications for toxic elements and also indicates the need for further specifications for aluminium, microbiological criteria and protein residues. This would apply for all food categories including those consumed by infants up to 16 weeks of age. The Panel further noted that no information was provided for oxidising enzymes. The Panel recommends that during the manufacturing process the oxidases and peroxidases present in acacia gum should be inactivated by heating to prevent the possible oxidative degradation of components in preparations to which acacia gum is added in line with earlier publications (Glicksman and Sand, 1973; Billaud et al., 1996; Ternes et al., 2007).

[15] Synonym for acacia gum, see Table 1.

According to Regulation (EC) No 1333/2008 (Annex III, part 5, section B), acacia gum (E 414) is authorised for use as a food additive in nutrient preparations intended to be used in foodstuffs for infants and young children, including in food for infants below 16 weeks of age. Dietary exposure to acacia gum (E 414) from its use as a food additive was assessed based on maximum carry over level set out in the EU legislation. The interested party confirmed that that the level of use of acacia gum (E 414) in infant formulae is compliant with this limit.

The exposure scenario is based on the consumption levels recommended in the relevant SC Guidance (EFSA Scientific Committee, 2017) to be used in risk assessment 200 and 260 mL formula[14]/kg bw per day as conservative mean and high level consumption values for 14 to 27 day old infants.

For infants below 16 weeks of age consuming infant formulae (FC 13.1.1) or infant food for special medical purpose (FSMP) (FC 13.1.5.1), mean exposure to acacia gum (E 414) was estimated to be 2 mg/kg bw per day while the high level was estimated at 2.6 mg/kg bw per day. Exposure estimates are based on the MPL for carry-over from nutrient formulations of 10 mg acacia gum (E 414)/kg in final foods for infants set in Annex III to Regulation (EC) No 1333/2008.

The interested parties did not submit toxicological and clinical data which can be used to assess the safety of the acacia gum in infants below the age of 16 weeks. In addition, post-marketing surveillance data were not provided. However, in this special situation, where the exposure is low and only due to the carry over, a MOS approach can be applied using available data from adult animals. Taking the highest doses tested without adverse effects in subchronic studies of 5,000 mg acacia gum/kg bw per day in rat and 20,000 mg acacia gum/kg bw per day in mice (EFSA ANS Panel, 2017) and comparing them with the exposure in infants of 2.6 mg/kg bw per day (high level estimate), the margins of safety (MOS) are roughly 2,000 and 8,000. These large MOS indicate that there is no reason for health concern. It is further noted that the data do not show genotoxicity (EFSA ANS Panel, 2017). Cases of allergenicity were not identified in the literature and in the former assessment (EFSA ANS Panel, 2017).

In Appendix A, the information sought by the call for data, the responses from interested parties and the results of the assessment from the Panel in the form of a comment are given.

4. Conclusions

Concerning the risk assessment for infants below 16 weeks of age, the Panel concluded that there is no reason for health concern considering the large MOS of roughly 2,000 and 8,000. These large MOS result from comparing the highest doses tested in rats and mice without adverse effects in subchronic studies with the high level estimate for the exposure of infants.

Concerning the follow-up on the former re-evaluation of acacia gum (E 414) as a food additive for all population groups (EFSA ANS Panel, 2017), the Panel noted that the specifications of acacia gum (E 414) for toxic elements, microbiological criteria and protein residues should be updated for all food categories. The Panel further noted that no information was provided by the interested parties for oxidising enzymes in the food additive and recommends that during the manufacturing process the oxidases and peroxidases present in acacia gum should be inactivated.

5. Recommendation

The Panel recommends:

- the European Commission considers lowering the limits in the specifications for acacia gum (E 414) for toxic elements and introducing specifications for aluminium, microbiological criteria and protein residues (see Table 2).
- the European Commission considers requiring that the oxidases and peroxidases in acacia gum (E 414) should be inactivated during the manufacturing process to avoid any oxidative degradation of components in preparations to which acacia gum (E 414) is added.

Documentation as provided to EFSA

1) Association for International Promotion of Gums (AIPG), 2019. Submission of data in response to the call for technical and toxicological data on acacia gum (E 414) for uses as a food additive in foods for all population groups including infants below 16 weeks of age. Submitted on March 2019.

2) DSM Nutritional Products Europe Ltd, 2019. Reply to the call for technical and toxicological data on acacia gum (E 414) for uses as a food additive in foods for all population groups including infants below 16 weeks of age. Submitted on March 2019.

References

Billaud C, Lecornu D and Nicolas J, 1996. Substrates and carboxylic acid inhibitors of partially purified polyphenol oxidase from gum arabic. Journal of Agriculture and Food Chemistry, 44, 1668–1675.

Bliss DZ, Jung HJ, Savik K, Lowry A, LeMoine M, Jensen L and Schaffer K, 2001. Supplementation with dietary fiber improves fecal incontinence. Nursing Research, 50, 203–213. https://doi.org/10.1097/00006199-200107000-00004

Binjumah M, Ajarem J, Ahmad M, 2018. Effects of the perinatal exposure of Gum Arabic on the development, behavior and biochemical parameters of mice offspring. Saudi Journal of Biological Sciences, 25(7), 1332–1338. https://doi.org/10.1016/j.sjbs.2016.04.008

Calame W, Weseler A, Viebke C, Flynn C and Siemensma A, 2008. Gum arabic establishes prebiotic functionality in healthy human volunteers in a dose-dependent manner. British Journal of Nutrition, 100, 1269–1275. https://doi.org/10.1017/S0007114508981447

EFSA (European Food Safety Authority), 2007. Opinion of the Scientific Committee related to uncertainties in dietary exposure assessment. EFSA Journal 2007;5(1):438, 54 pp. https://doi.org/10.2903/j.efsa.2007.438

EFSA (European Food Safety Authority), 2008. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) - Safety of aluminium from dietary intake. EFSA Journal 2008;6(7):754, 34 pp. https://doi.org/10.2903/j.efsa.2008.754

EFSA (European Food Safety Authority), 2009. Scientific opinion of the Panel on Contaminants in the Food Chain - Cadmium in food. EFSA Journal 2009;7(3):980, 139 pp. https://doi.org/10.2903/j.efsa.2009.980

EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), Mortensen A, Aguilar F, Crebelli R, Di Domenico A, Frutos MJ, Galtier P, Gott D, Gundert-Remy U, Lambré C, Leblanc J-C, Lindtner O, Moldeus P, Mosesso P, Oskarsson A, Parent-Massin D, Stankovic I, Waalkens-Berendsen I, Woutersen RA, Wright M, Younes M, Brimer L, Christodoulidou A, Lodi F, Tard A and Dusemund B, 2017. Scientific Opinion on the re-evaluation of acacia gum (E 414) as a food additive. EFSA Journal 2017;15(4):4741, 51 pp. https://doi.org/10.2903/j.efsa.2017.4741

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2009. Scientific opinion on Arsenic in food. EFSA Journal 2009;7(10):1351, 199 pp. https://doi.org/10.2903/j.efsa.2009.1351

EFSA CONTAM Panel (Panel on Contaminants in the Food Chain), 2010. Scientific opinion on Lead in food. EFSA Journal 2010;8(4):1570, 151 pp. https://doi.org/10.2903/j.efsa.2010.1570

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2012. Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. EFSA Journal 2012;10(12):2985, 15 pp. https://doi.org/10.2903/j.efsa.2012.2985

EFSA 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(11):1051, 22 pp. https://doi.org/10.2903/j.efsa.2009.1051

EFSA Scientific Committee, 2012. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 2012;10(3):2579, 32 pp. https://doi.org/10.2903/j.efsa.2012.2579

EFSA Scientific Committee, 2017. Guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age. EFSA Journal 2017;15(5):4849, 58 pp. https://doi.org/10.2903/j.efsa.2017.4849

European Commission, online. Approach for the follow-up of EFSA's scientific opinions on the re-evaluation of the safety of permitted food additives for which some concerns have been identified. Available online: https://ec.europa.eu/food/sites/food/files/safety/docs/fs_food-improvement-agents_reeval_approach.pdf

Glicksman M and Sand RE, 1973. Gum Arabic. In: Whistler RL (ed.). Industrial Gums. Polysaccharides and Their Derivatives. Academic Press, NY. pp. 197–265.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1982. Toxicological evaluation of certain food additives, Series number 17, ID number 526. Arabic gum (gum arabic). Twenty-sixth Report of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additive Series 17. Available online: http://www.inchem.org/documents/jecfa/jecmono/v17je06.htm

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1990. Toxicological evaluation of certain food additives and contaminants, Series number 26, ID number 686. Gum arabic. Thirty-fifth Report of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additive Series 26. Available online: http://www.inchem.org/documents/jecfa/jecmono/v26je07.htm

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2000. Guidelines for the preparation of toxicological working papers for the Joint FAO/WHO Expert Committee on Food Additives. Geneva, Switzerland.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2006. Gum Arabic. Joint FAO/WHO Expert Committee on Food Additives. Available online: http://www.fao.org/ag/agn/jecfa-additives/details.html?id=766

SCF (Scientific Committee for Food), 1991. Reports of the Scientific Committee for Food (24th Series). First Report of the Scientific Committee for Food on the essential requirements for weaning foods. Opinion expressed on 30 March 1990. Food science and techniques. Available online: http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_24.pdf

SCF (Scientific Committee on Food), 1998. Scientific opinion on the applicability of the ADI (Acceptable Daily Intake) for food additives to infants (expressed on 17/09/1998). Available at: https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_out13_en.pdf

SCF (Scientific Committee for Food), 1999. Reports of the Scientific Committee for Food (43rd Series). Opinion on certain additives for use in foods for infants and young children in good health and in foods for special medical purposes for infants and young children. Opinion expressed on 13 June 1997. Food science and techniques. Available online: http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_43.pdf

TemaNord, 2002. Acacia gum. In: Food additives in Europe 2000 - Status of safety assessments of food additives presently permitted in the EU. Available online: http://www.norden.org/en/publications/publikationer/2002-560/at_download/publicationfile

Ternes W, Taeufel A, Tunger L and Zobel M, 2007. Gummi Arabicum in Lexikon der Lebensmittel und der Lebensmittelchemie. Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, Germany. pp. 747–748.

Glossary and/or abbreviations and/or acronyms

ADI	acceptable daily intake
ANS Panel	EFSA Panel on Food Additives and Nutrient Sources added to Food
APC	aerobic plate count
BMDL	Benchmark dose level
bw	body weight
CAS	Chemical Abstract Service
EINECS	European Inventory of Existing Commercial Substances
CFU	colony forming unit
FAF Panel	EFSA Panel on Food Additives and Flavourings
FAO/WHO	Food and Drug Organization/World Health Organization
FC	food category
FSMP	food for special medical purposes
GD	gestation day
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LOD	limit of detection
Mintel	GNPD Mintel's Global New Products Database
MOE	margin of exposure
MOS	margin of safety
MPL	maximum permitted levels
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
PND	postnatal day
SC	Scientific Committee of EFSA
SCF	Scientific Committee on Food
TAMC	total anaerobic microbial count
TWI	tolerable weekly intake
TYMC	total combined yeast and mould count

Appendix A – Data requested in the call for data (Call for technical and toxicological data on acacia gum (E 414) for uses as a food additive in foods for all population groups including infants below 16 weeks of age.[16]

Kind of data	Data requested in the call for data	Responses from interested parties	Comment
A. Information regarding the follow-up of the conclusions and the recommendations of the EFSA ANS Panel opinion on the safety of acacia gum (E 414) as a food additive			
1. Technical data	Analytical data on current levels of aluminium, lead, mercury, cadmium and arsenic in commercial samples of the food additive	Received	Used in the proposal for new specifications
	The lowest technologically achievable level for aluminium, lead, mercury, cadmium, and arsenic in order to adequately define their maximum limits in the specifications	Received	Not accepted, recommendation to lower the levels
	Current levels of residual proteins and oxidising enzymes (oxidases and peroxidases) in acacia gum (E 414) preparations	1) Protein: received 2) Oxidising enzymes (oxidases and peroxidases): not received	1) Added to the specifications, recommendation 2) Recommendation to inactivate the enzymes; to introduce residual limits
	Data demonstrating the lowest total aerobic microbial count (TAMC) and total combined yeast and mould count (TYMC) that can be achieved	Received	Included into the new specifications, recommendation
2. Literature searches	Literature searches	Received	Assessed, no further follow-up

[16] Available from: https://www.efsa.europa.eu/en/consultations/call/181010-4 and responses from interested parties.

Kind of data	Data requested in the call for data	Responses from interested parties	Comment
B. Information required for the risk assessment of acacia gum (E 414) as a food additive for use in foods for infants below 16 weeks of age			
1. Technical data	Information on the resulting concentrations of acacia gum (E 414), alone or in combination with other thickening agents (indication of food additive name and resulting concentration) in these foods	Not received	Assessed, no further follow-up
	Information on the fate and the reaction products of acacia gum (E 414) in these food categories	Received	Assessed, no further follow-up
	Information on particular specification requirements for identity and the purity of acacia gum (E 414) to be used in these food categories (e.g. content residual proteins and enzymes, toxic elements). Analytical data on impurities in the final foods for infants below 16 weeks of age need to be provided when no legal limit has been established	Not provided	Assessed, no further follow-up
	Data demonstrating the absence of *Cronobacter* (Enterobacter) *sakazakii* in the food additive	Received	Included into the new specifications, recommendation
2. Toxicological data	Clinical data focusing on gastrointestinal effects to assess the safety of acacia gum (E 414) when present in foods for infants below 16 weeks of age	No data received	Assessed, no further follow-up
	Post-marketing surveillance reports on undesired and adverse reactions (including e.g. flatulence, gastrointestinal discomfort, changes of stool-frequencies and -consistency, diarrhoea and allergic reactions), indicating the ages and other relevant data of the exposed infants and young children and the use level of acacia gum (E 414) in the marketed products	No data received	Assessed, no further follow-up
3. Literature searches	Literature searches should be conducted relevant for the safety evaluation of acacia gum (E 414) when used in foods for infants below 16 weeks of age up to the date of the data submission, as described in the Guidance for submission for food additive evaluations (Section 5.3)	Received	Assessed, no further follow-up

Safety Assessment of Acacia Senegal Gum and Acacia Senegal Gum Extract as Used in Cosmetics

Status:	Re-Review for Panel Consideration
Release Date:	August 18, 2023
Panel Meeting Date:	September 11-12, 2023

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Thomas J. Slaga, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D., and the Senior Director is Monice Fiume. This report was prepared by Regina Tucker, M.S., Scientific Analyst/Writer, CIR.

1620 L Street, NW, Suite 1200 ◇ Washington, DC 20036-4702 ◇ ph 202.331.0651 ◇
cirinfo@cir-safety.org

Commitment & Credibility since 1976

Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Regina Tucker, MS
Scientific Analyst/Writer, CIR
Date: August 18, 2023
Subject: Re-Review of the Safety Assessment of Acacia Senegal Gum and Acacia Senegal Gum Extract

The Expert Panel for Cosmetic Ingredient Safety (Panel) previously reviewed the safety of Acacia Senegal Gum and Acacia Senegal Gum Extract as part of a larger group of ingredients derived from the acacia plant. In 1998, the Panel initially issued a final report with an insufficient data conclusion for the entire group of acacia ingredients reviewed at that time, including Acacia Senegal Gum and Acacia Senegal Gum Extract. Subsequently, the Panel's data needs were met for only Acacia Senegal Gum and Acacia Senegal Gum Extract, and an amended final report was published in 2005. At that time, the Panel concluded that Acacia Senegal Gum and Acacia Senegal Gum Extract are safe as used in cosmetic products. The published amended final report (which includes all information from the original report, as well as the data that were submitted in response to the original insufficient data conclusion) is included for your use (identified as *amendedreport_Acacia_092023*). Please note that because the other ingredients included in the larger group had an insufficient data conclusion, they are not included in this rereview.

Because it has been at least 15 years since the previous safety assessment was published, in accordance with Cosmetic Ingredient Review (CIR) Procedures, the Panel should consider whether the safety of Acacia Senegal Gum and Acacia Senegal Gum Extract should be reconsidered. In June 2023, an extensive search of the world's literature was performed for studies dated 2000 forward. (Please note that since the 2005 amended report also identifies gum arabic as a technical name for Acacia Senegal Gum, in an effort to provide the Panel with the most accurate and up-to-date information on these ingredients, the term gum arabic was also a part of this search.) An historical overview, comparison of original and new use data, the search strategy used, and a synopsis of notable new data are enclosed herein (*newdata_Acacia_092023*).

New non-cosmetic use data that consists of current European Union regulations regarding use as a food additive were identified. Data which cover reproductive toxicity and occupational case studies were also found and are included for your review.

Also included for your review are current and historical use data (*usetable_Acacia_092023*). The frequency and concentration of use of Acacia Senegal Gum and Acacia Senegal Gum Extract has increased for both ingredients since the amended final report was published in 2005, with significant increases noted for Acacia Senegal Gum. Acacia Senegal Gum is now reported to be used in 287 formulations at up to 26.7% in other oral hygiene products (this category of use was not previously report), and therefore can be incidentally ingested; in 2001, it was reported to be used in 1 formulation and at up to 9%. Use in baby products is also now reported in the VCRP. The Panel should note that upon reviewing the use section in the 2005 report, a discrepancy was found between the highest concentration of use reported in the text (shampoo, 9%) and the highest concentration reported in the table (mascara, 3-9%). The value presented in this re-review reflects the reported value as it appears both in the abstract and use table presented in the 2005 amended safety assessment.

If upon review of the new studies and updated use data the Panel determines that a re-review is warranted, a Draft Amended Report will be presented at an upcoming meeting.

Re-Review – Acacia Senegal Gum & Gum Extract - History and New Data

(Regina Tucker – September 2023 meeting)

Ingredients (2)	Citation	Conclusion	Use - New Data	Use - Historical Data	Notes
Acacia Senegal Gum **Acacia Senegal Gum Extract** *Acacia Senegal Gum and Acacia Senegal Gum Extract were previously reviewed as part of a larger group of ingredients derived from the acacia plant. The data needs were only met for those two ingredients. All of the other ingredients were still insufficient and are not included in this rereview.*	IJT 24(S3):75-118, 2005	Safe as used	Acacia Senegal Gum frequency of use (2023): 287 conc of use (2022): 0.00001-26.7% Acacia Senegal Gum Extract frequency of use (2023): 9 con of use (2023) 0.001-3%	Acacia Senegal Gum frequency of use (2001): 1 conc of use (2001): 0.0001-9% Acacia Senegal Gum Extract frequency of use (2001): no reported use concentration of use (2001): 0.001%	Significant increase in frequency of use of Acacia Senegal Gum. The maximum concentration of use of both ingredients has increased. Significant increases in frequency of use in eye make-up preparations. New use in the following categories: Baby products, bath preparations, and oral hygiene products.

NOTABLE NEW DATA			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
Acacia Senegal Gum			
Non-Cosmetic Use			
EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) Re-evaluation of acacia gum (E 414) as a food additive. EFSA J 2017; 15(4): 04741.	Re-evaluation on the safety of Acacia Senegal Gum as a food additive, Europe	The Panel on Food Additive and Nutrient Sources added to Food concluded that there is no need for a numerical acceptable daily intake value, and there is no safety concern for the general population as a food additive.	This citation provides up-to-date EU regulatory information that is not included in the original report.
EFSA Panel on Food Additives and Flavorings (FAF) Opinion on the re-evaluation of acacia gum (E 414) as a food additive in foods for infants below 16 weeks of age and the follow-up of its re-evaluation as a food additive for uses in foods for all population groups. EFSA J. 2019;17(12):05922.	Review of the re-evaluation and safety of Acacia Senegal Gum (acacia gum) in food for infants below 16 wk of age.	The Panel on Food Additives and Flavorings concluded for infants below 16 weeks of age, there is no reason for health concern. The Panel also noted that for the general population specifications for toxic elements, microbiological criteria, and protein residues should be updated for all food categories and that during the manufacturing process the oxidases and peroxidases present in Acacia Senegal Gum should be inactivated.	This citation provides up-to-date EU regulatory information that is not included in the original report.
EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP); Safety and efficacy of a feed additive consisting of acacia gum (Gum arabic) for all animal species (A.I.P.G. Association for International Promotion of Gums). EFSA J. 2022 Apr 29;20(4)	Opinion on the safety and efficacy of Gum Arabic as a feed additive for all animal species	Acacia gum is safe up to approximately 280 mg/kg complete feed for chickens for fattening, 375 mg/kg complete feed for turkeys for fattening, 400 mg/kg complete feed for rabbit, 500 and 600 mg/kg complete feed for piglets and pigs for fattening, respectively, 1100 mg/kg complete feed for cattle for fattening and 1250 mg/kg complete feed for veal calves and salmonids. The use of the additive in animal nutrition is considered safe for the consumer and the environment. Acacia gum is a potential dermal and respiratory sensitizer. No conclusion can be reached on the irritant potential for skin or eye.	This citation provides up-to-date EU regulatory information that is not included in the original report.

NOTABLE NEW DATA			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
Toxicological Studies			
Doi Y, Ichihara T, Hagiwara A, Imai N, Tamano S, Orikoshi H, Ogasawara K, Sasaki Y, Nakamura M, Shirai T. A ninety-day oral toxicity study of a new type of processed Gum arabic, from Acacia tree (Acacia senegal) exudates, in F344 rats. Food Chem Toxicol. 2006 Apr;44(4):560-6.	Subchronic Toxicity	F344/DuCrj rats of both sexes (10 rats/sex/group) were administered diets containing the new generation of Acacia Senegal Gum at doses of 0 (control), 1.25, 2.5, and 5% for 90 d. No deaths were observed in any groups during the 90 d of feeding and the treatment had no effects on clinical signs, body weights, and food and water consumption, or on findings of urinalysis, ophthalmology, hematology, or blood biochemistry. Gross pathology and histopathology exhibited no differences of toxicological significance between control and treated rats. Increased relative cecum (filled) weights, evident in both sexes of the 5.0% group and females of 1.25 and 2.5% groups, were considered to be a physiological adaptation. The toxic level of the new generation of Acacia Senegal Gum is no more than 5.0%. The no observed adverse effect level (NOAEL) was concluded to be 5.0% (3117 mg/kg bw/d for males, and 3296 mg/kg bw/d for females) from the present study.	This study provides details on a new generation of Acacia Senegal Gum that are not provided in the original report.
Developmental and Reproductive Toxicity (DART)			
Nasir O, Alqadri N, Elsayed S, Ahmed O, Alotaibi SH, Baty R, Omer H, Abushal SA, Umbach AT. Comparative efficacy of Gum arabic (*Acacia senegal*) and *Tribulus terrestris* on male fertility. Saudi Pharm J. 2020 Dec;28(12):1791-1796.	DART-Oral	Adult male mice Balb/c were divided into 9 groups (1 male, 2 females/ group) and were given 0 (controls; given normal tap water) or 5% (w/v) Acacia Senegal Gum (powder) dissolved in tap water for 21 d. The group treated with Acacia Senegal Gum produced more offspring as well as a higher number of living offspring when compared to control. Testosterone levels in males were also increased when compared to the control group. Histopathological analysis showed the 5% Acacia Senegal Gum had normal seminiferous tubules with increase spermatogenesis.	No, study provides information on spermatogenesis and effects on offspring production. Similar to Huynh et al. 2000.
Nofal AE, Okdah YA, Rady MI, Hassaan HZ. Gum Acacia attenuates cisplatin toxic effect spermatogenesis dysfunction and infertility in rats. Int J Biol Macromol. 2023; 240:124292.	DART-oral	Sprague Dawley rats were separated into groups of 10 rats each and were given (1) no treatment, (2) 7.5 mg/kg bw/d Acacia Senegal Gum via stomach tube, (3) 7.5 mg/kg bw/w cisplatin, and (4) both Acacia Senegal Gum and cisplatin for 4 wk. The rats that received Acacia Senegal Gum showed little changes when compared to controls in terms of seminiferous tubule diameter, epithelial height, Johnsen testicular score, or Cosentino's scores. Rats treated with only cisplatin showed a decrease seminiferous tubule diameter, decreased seminiferous tubule epithelial height, decreased Johnsen testicular score, and an increased Cosentino's score. Co-administration of Acacia Senegal Gum with cisplatin mitigated the dysfunction in spermatogenesis and reversed testicular damage caused by cisplatin. Co-administration with cisplatin also improved the following endpoints: levels of testosterone and luteinizing hormone in blood sera, histometric measurements of seminiferous tubules diameter, and spermatogenesis.	The study provides information on spermatogenesis but also provides data on co-administration of Acacia Senegal Gum and cisplatin.

NOTABLE NEW DATA			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
Anti-Carcinogenicity			
Avelino ALN, Silva NVRE, Oliveira GB, Silva AAS, Cavalcanti BC, Jamacaru FVF, Dornelas CA. Antioxidant and Antigenotoxic Actions of Gum arabic on the Intestinal Mucosa, Liver and Bone Marrow of *Swiss* Mice Submitted to Colorectal Carcinogenesis. Nutr Cancer. 2022;74(3):956-964.	Effects on colorectal carcinogenesis	Swiss male mice were induced with colorectal carcinogenesis. Three control groups (Groups I-III) were formed (6 mice each) and given an intraperitoneal (i.p.) injection of saline solution. Three experimental groups (Group IV -12 mice, Group V -12 mice and Group VI -13 mice) were also formed and given azoxymethane at 10 mg/kg i.p. In the weeks that followed the animals received daily administration by gavage of 5 ml/kg of water (Groups I and IV), 2.5% Acacia Senegal Gum (Groups II and V) or 5% Acacia Senegal Gum (Groups III and VI). All organs were evaluated for tumors. Treatment with Acacia Senegal Gum at concentrations of 2.5% and 5% reduced the formation of aberrant crypts, aberrant crypts foci and aberrant crypt foci with fewer than 5 crypts in the colon of the mice. A similar effect was observed in the liver and bone marrow.	Yes, anti-carcinogenicity studies are not in the current report.
Nasir O, Wang K, Föller M, Bhandaru M, Sandulache D, Artunc F, Ackermann TF, Ebrahim A, Palmada M, Klingel K, Saeed AM, Lang F. Downregulation of angiogenin transcript levels and inhibition of colonic carcinoma by Gum arabic (Acacia senegal). Nutr Cancer. 2010;62(6):802-10.	Effects on colonic carcinoma-	BALB/c WT mice of either sex were divided into 4 groups of 12 animals each. One group underwent carcinogenic treatment via 20 mg/kg 1,2-dimethylhydrazine (DMH) intraperitoneally followed by 3 cycles of distilled water containing 30 g/l synthetic dextran sulfate sodium (DSS) for 7 d, while another group underwent carcinogenic treatment and was given Acacia Senegal Gum (100 g/l in distilled water). Group 3 underwent an i.p. injection of 20 mg/kg sodium chloride and was treated with Acacia Senegal Gum, and group 4 underwent the same injection with sodium chloride. In the second, experiment 8 BALB/c mice underwent the same carcinogenic treatment and were given Acacia Senegal Gum, except during periods of DMH/DSS treatment. 8 BALB/c mice did not receive treatment. In the third experiment, 8 BALB/c mice underwent Acacia Senegal Gum treatment after the completion of the DMH and DSS treatment to investigate the effect of Acacia Senegal Gum on tumor promotion. The other 8 BALB/c mice did not receive treatment. Ingestion of Acacia Senegal Gum decreased transcript levels of angiogenetic factors angiotensin 1 (78 ± 18%), 3 (88 ± 15%), and 4 (92 ± 13%) within 4 d. Acacia Senegal Gum also reduced the number of tumors by 60%. Weight gain was slightly smaller in mice treated with Acacia Senegal Gum and in mice undergoing chemical carcinogenesis. Weight gain was significantly less in mice treated with DMH/DSS and Acacia Senegal Gum than in mice treated with only DMH/DSS.	Yes, anti-carcinogenicity studies are not in the current report.
Clinical Studies			
Tschannen MP, Glück U, Bircher AJ, Heijnen I, Pletscher C. Thaumatin and Gum arabic allergy in chewing gum factory workers. Am J Ind Med. 2017 Jul;60(7):664-669.	Case Report/Occupational Exposure	Eight male employees aged 23-52 yr (average age 36 yr) were exposed to a powder mixture composed of 10% thaumatin and 90% Acacia Senegal Gum, which led to allergic symptoms in the upper airways. Skin prick tests (SPT) were performed. Anterior rhinoscopy was used to assess the state of the turbinates. A positive SPT for pure thaumatin was obtained in all 4 individuals with rhinitis of whom also had a positive skin prick test result for pure Acacia Senegal Gum and Acacia Senegal Gum specific IgE.	

NOTABLE NEW DATA			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
Sander I, Raulf-Heimsoth M, Wiemer K, Kespohl S, Brüning T, Merget R. Sensitization due to Gum arabic (Acacia senegal): the cause of occupational allergic asthma or crossreaction to carbohydrates? Int Arch Allergy Immunol. 2006;141(1):51-6.	Case Report/Occupational Exposure	A 30-yr-old male pharmaceutical industry worker was admitted for medical advice after experiencing workplace-related shortness of breath, chest tightness, runny nose, itching, swelling, redness of the eyes and redness of the face and neck. Symptoms occurred mainly during weighing Acacia Senegal Gum or talc at his workplace. The case was studied further to identify IgE-binding components responsible for the work-related symptoms. The study showed that Acacia Senegal Gum may cause occupational allergic rhinitis and asthma with urticaria symptoms in some patients. One hundred nineteen patients underwent SPT with Acacia Senegal Gum (1% w/v, protein concentration 40 ug/ml; material from the patients' workplace) and environmental allergens. Thirty-six subjects with total IgE ≥ 100 kU/l or at least 1 positive SPT were tested for IgE to Acacia Senegal Gum. Additionally, the sera of 7 highly atopic patients without occupational exposure to Acacia Senegal Gum were selected to complete the control group for in vitro tests. Only 3 subjects showed IgE to Acacia Senegal Gum: one control with positive SPT, one control with negative SPT, and the patient that came to the clinic.	No occupational exposure on Acacia Senegal Gum was provided in original report.
Viinanen A, Salokannel M, Lammintausta K. Gum arabic as a cause of occupational allergy. J Allergy (Cairo). 2011; 2011:841508.	Occupational Exposure	Eleven candy factory workers with respiratory and/or skin symptoms were referred to the hospital. The workers reported some of the following symptoms: hives, erythema of the hands, dyspnea, rhinitis, eye symptoms, redness of the skin, itching of the skin, cough, nasal congestion, and secretion. Six candy factory workers had occupational allergic disease, in which 4 of the cases were confirmed to be occupational asthma caused by Acacia Senegal Gum with contact urticaria. Contact urticaria was verified in 2 of the workers via cutaneous exposure test. One worker underwent a specific bronchial provocation test to Acacia Senegal Gum and was found to be positive.	No occupational exposure on Acacia Senegal Gum was provided in original report.
Romita, P., Bufano, T., Antelmi, A., Gelardi, M. and Foti, C. (2018), Occupational allergic rhinitis and contact urticaria caused by Gum arabic in a candy factory worker. Contact Dermatitis, 78: 427-428.	Occupational Exposure	A 35-yr-old man presented with a 5-yr history of recurrent bilateral nasal obstruction; since the previous year, it had been followed by the onset of wheals on his arms. His job involved making candies with Acacia Senegal Gum. The patient denied symptoms related to ingestion of Acacia Senegal Gum. SPTs were performed with 10% wt/vol Acacia Senegal Gum in physiological saline, yielding a 9-mm wheal. Open patch testing was performed by applying 10% wt/vol Acacia Senegal Gum in saline solution on his back and leaving it under occlusion for 20 min; this also produced multiple wheals. ImmunoCAP tests resulted in positive results at a level of 0.33 kUA/l. The level of total IgE was 85 kU/l. A nasal provocation test with (200 AU/ml) gave a positive result.	No occupational exposure on Acacia Senegal Gum was provided in original report.

Abbreviations: DMH - dimethylhydrazine; DSS - dextran sulfate sodium; i.p. - intraperitoneal; kUA/l-kilo units of antibody per liter; NOAEL-no observed adverse effect level SPT- skin prick test

Search (from 2000 to present)

PubMed

(((“Acacia Senegal Gum”) OR (9000-01-5[CAS No.])) AND (("2000"[Date - Publication]: "3000"[Date – Publication]))) – 83 hits; 9 useful hits

(((“Acacia Senegal Gum Extract”-11”) OR (9000-01-5[CAS No.])) AND (("2000"[Date - Publication]: "3000"[Date – Publication]))) – 83 hits; 9 useful hits

(((“Gum arabic”) OR (9000-01-5[CAS No.])) AND (("2000"[Date - Publication]: "3000"[Date – Publication]))) – 1,721;7 useful hit

The following qualifiers were used in the search of Gum arabic: Absorption, Acute, Allergy, Allergic, Allergenic, Cancer, Carcinogen, Chronic, Development, Developmental Excretion, Genotoxic, Irritation, Metabolism, Mutagen, Mutagenic, Penetration, Percutaneous, Pharmacokinetic, Repeated dose, Reproduction, Reproductive, Sensitization, Skin, Subchronic, Teratogen, Teratogenic, Toxic, Toxicity, Toxicokinetic, Toxicology, Tumor.

Table 1. Frequency (2023/2001) and concentration (2022/2000) of use according to likely duration and exposure and by product category

	Acacia Senegal Gum				Acacia****				Acacia Senegal Gum Extract			
	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2023[1]	2001[2]	2022[3]	2000[2]	2023[1]	2001[2]	2022[3]	2000[2]	2023[1]	2001[2]	2022[3]	2000[2]
Totals*	**287**	**1**	**0.00001-26.7**	**0.0001-9**	**8**	**33**	****	****	**9**	**NR**	**0.001-3**	**0.001**
summarized by likely duration and exposure**												
Duration of Use												
Leave-On	*210*	*1*	*0.00001-3.8*	*0.0001-9*	*4*	*27*	****	****	*5*	*NR*	*0.001-3*	*NR*
Rinse-Off	*66*	*NR*	*0.0015-26.7*	*NR*	*4*	*5*	****	****	*4*	*NR*	*NR*	*0.001*
Diluted for (Bath) Use	*11*	*NR*	*NR*	*NR*	*NR*	*1*	****	****	*NR*	*NR*	*NR*	*NR*
Exposure Type												
Eye Area	80	1	2-3.8	1-9	2	20	****	****	1	NR	3	NR
Incidental Ingestion	2	NR	0.24-26.7	NR	1	1	****	****	NR	NR	NR	NR
Incidental Inhalation-Spray	57[a], 44[b]	NR	0.003[a]	0.0001[a]	1[c]	1; 3[b]	****	****	3[b]	NR	NR	NR
Incidental Inhalation-Powder	4; 44[b]; 3[c]	NR	0.00001-0.03[c]	0.5	NR	3[b]	****	****	3[b]	NR	0.4[c]	NR
Dermal Contact	200	NR	0.00001-3.4	0.02-3	3	9	****	****	6	NR	0.4	0.001
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	****	****	NR	NR	NR	NR
Hair - Non-Coloring	14	NR	0.003-0.61	0.0001	NR	NR	****	****	1	NR	NR	NR
Hair-Coloring	NR	NR	NR	NR	3	5	****	****	1	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	****	****	NR	NR	0.001	NR
Mucous Membrane	43	NR	0.0015-26.7	NR	1	2	****	****	1	NR	NR	0.001
Baby Products	3	NR	NR	NR	NR	NR	****	****	NR	NR	NR	NR
as reported by product category												
Baby Products												
Baby Lotions/Oils/Powders/Creams	3	NR	NR	NR								
Bath Preparations (diluted for use)												
Bath Oils, Tablets, and Salts	10	NR	NR	NR								
Bubble Baths	1	NR	NR	NR								
Other Bath Preparations					NR	1	****	****				
Eye Makeup Preparations												
Eyebrow Pencil	NR	NR	NR	1								
Eyeliner	1	NR	NR	3								
Eye Shadow	1	NR	NR	NR	1	NR	****	****				
Eye Lotion	1	NR	NR	NR								
Mascara	71	1	2-3.8	3-9***	NR	18	****	****	1	NR	3	NR
Other Eye Makeup Preparations	6	NR	NR	NR	1	2	****	****				
Fragrance Preparations												
Powders (dusting/talcum, excl aftershave talc)	NR	NR	NR	0.5								
Hair Preparations (non-coloring)												
Hair Conditioner	2	NR	0.003	NR	1	NR	****	****				
Hair Straighteners	1	NR	NR	NR								
Rinses (non-coloring)					1	NR	****	****				
Shampoos (non-coloring)	5	NR	0.61	NR***	1	NR	****	****	1	NR	NR	NR
Tonics, Dressings, and Other Hair Grooming Aids	4	NR	0.003	0.0001								
Other Hair Preparations	2	NR	NR	NR								

Table 1. Frequency (2023/2001) and concentration (2022/2000) of use according to likely duration and exposure and by product category

	Acacia Senegal Gum				Acacia****				Acacia Senegal Gum Extract			
	# of Uses		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*	
	2023[1]	**2001[2]**	**2022[3]**	**2000[2]**	**2023[1]**	**2001[2]**	**2022[3]**	**2000[2]**	**2023[1]**	**2001[2]**	**2022[3]**	**2000[2]**
Hair Coloring Preparations												
Hair Tints					4	1	****	****				
Hair Rinses (coloring)					2	NR	****	****	1	NR	NR	NR
Hair Color Sprays (aerosol)					4	1	****	****				
Other Hair Coloring Preparation					2	3	****	****				
Makeup Preparations												
Blushers (all types)	1	NR	NR	NR								
Face Powders	4	NR	NR	NR								
Foundations	6	NR	NR	NR	NR	1	****	****				
Leg and Body Paints	1	NR	NR	NR								
Lipstick					NR	1	****	****				
Other Makeup Preparations	1	NR	0.03	NR	NR	1	****	****				
Manicuring Preparations (Nail)												
Nail Polish and Enamel	1	NR	NR	NR	1	NR	****	****				
Other Manicuring Preparations									NR	NR	0.001	NR
Oral Hygiene Products												
Dentifrices	NR	NR	0.24-2.9	NR								
Other Oral Hygiene Products	2	NR	1.9-26.7	NR	1	NR	****	****				
Personal Cleanliness Products												
Bath Soaps and Detergents	26	NR	0.0075-0.61	NR					NR	NR	NR	0.001
Other Personal Cleanliness Products	4	NR	0.0015	NR					1	NR	NR	NR
Shaving Preparations												
Other Shaving Preparations	1	NR	NR	NR								
Skin Care Preparations												
Cleansing	12	NR	0.003	NR					1	NR	NR	NR
Face and Neck (exc shave)	43	NR	0.03 (not spray)	NR					3	NR	0.4 (not spray)	NR
Body and Hand (exc shave)	1	NR	0.00001-.0075 (not spray)	NR	1	3	****	****				
Moisturizing	46	NR	0.006-0.0075 (not spray)	NR								
Night	6	NR	0.5 (not spray)	NR								
Paste Masks (mud packs)	13	NR	NR	NR	NR	1	****	****				
Other Skin Care Preparations	11	NR	3.4	0.02					1	NR	NR	NR
Suntan Preparations												
Suntan Gels, Creams, and Liquids	1	NR	0.03 (not spray)	NR								

NR – not reported

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

**likely duration and exposure are derived based on product category (see Use Categorization https://www.cir-safety.org/cir-findings)

***The value presented here reflects the reported value as it appears use table in the 2005 amended safety assessment; a discrepancy exists in the text of that report.

**** According to the *Dictionary*, Acacia is a technical name for Acacia Senegal Gum, and therefore, the frequency of use for Acacia has been included here; concentration of use data for that name were not obtained in 2000 or 2022.

[a] It is possible these products are sprays, but it is not specified whether the reported uses are sprays.

[b] Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, therefore the information is captured in both categories.

[c] It is possible these products are powders, but it is not specified whether the reported uses are powders.

International Journal of Toxicology, 24(Suppl. 3):75–118, 2005

ISSN: 1091-5818 print / 1092-874X online
DOI: 10.1080/10915810500257170

Final Report of 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[1]

These ingredients are derived from various species of the acacia plant. Only material derived from *Acacia senegal* are in current use according to industry data. The concentration at which these ingredients are reported to be used ranges from 9% in mascara to 0.0001% in tonics, dressings, and other hair-grooming aids. Gum arabic is a technical name for Acacia Senegal Gum. Gum arabic is comprised of various sugars and glucuronic acid residues in a long chain of galactosyl units with branched oligosaccharides. Gum arabic is generally recognized as safe as a direct food additive. Little information is available to characterize the extracts of other Acacia plant parts or material from other species. Acacia Concinna Fruit Extract was generally described as containing saponins, alkaloids, and malic acid with parabens and potassium sorbate added as preservatives. Cosmetic ingredient functions have been reported for Acacia Decurrens Extract (astringent; skin-conditioning agent—occlusive) and Acacia Farnesiana Extract (astringent), but not for the other Acacias included in this review. Toxicity data on gum arabic indicates little or no acute, short-term, or subchronic toxicity. Gum arabic is negative in several genotoxicity assays, is not a reproductive or developmental toxin, and is not carcinogenic when given intraperitoneally or orally. Clinical testing indicated some evidence of skin sensitization with gum arabic. The extensive safety test data on gum arabic supports the safety of Acacia Senegal Gum and Acacia Senegal Gum Extract, and it was concluded that these two ingredients are safe as used in cosmetic formulations. It was not possible, however, to relate the data on gum arabic to the crude Acacias and their extracts from species other than *Acacia senegal*. Therefore, the available data were considered insufficient to support the safety 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, and Acacia Senegal Extract in cosmetic products. The additional data needed to complete the safety assessment for these ingredients include (1) concentration of use; (2) identify the specific chemical constituents, and clarify the relationship between crude Acacias and their extracts and the Acacias and their extracts that are used as cosmetic ingredients; (3) data on contaminants, particularly relating to the presence of pesticide residues, and a determination of whether Acacia melanoxylon is used in cosmetics and whether acamelin (a quinone) and melacacidin (a flavin) are present in the Acacias that are being used; (4) skin sensitization study (i.e., dose response to be determined); (5) contact urticaria study at use concentration; and (6) ultraviolet (UV) absorption spectrum; if there is significant absorbance in the UVA or UVB range, then a photosensitization study may be needed. It was also noted that other data may be needed after clarification of the chemical constituents of the Acacia-derived ingredients.

INTRODUCTION

The Cosmetic Ingredient Review (CIR) Expert Panel began developing a safety assessment of Acacia-derived ingredients in 1996. In 1998, a final safety assessment was issued with the conclusion that the available data were not sufficient to support the safety of these ingredients in cosmetics. The needed data included concentration of use; specific chemical constituents, including the relationship between crude Acacias and their extracts and the Acacias and their extracts that are used as cosmetic ingredients; contaminants, particularly relating to the presence of pesticide residues and a determination if Acacia melanoxylon is used in cosmetics, and whether acamelin (a quinone) and melacacidin (a flavin) are present in the Acacias that are being used; skin sensitization dose response; contact urticaria data use concentration; and ultraviolet (UV) absorption; if there is significant absorbance in the UVA or UVB range, then a photosensitization study may be needed. The Panel noted that other studies may be requested after clarification of the chemical constituents of the Acacias.

In 2000 and 2001, new data were received including use concentration data on Acacia Senegal and Acacia Senegal Extract;

Received 15 April 2005; accepted 12 July 2005.

Address correspondence to Wilbur Johnson, Cosmetic Ingredient Review, at CIR, 1101 17th Street, NW, Suite 310, Washington, DC 20036, USA. E-mail: andersena@cir-safety.org

[1]Reviewed by the Cosmetic Ingredient Review Expert Panel. This report was prepared by Wilbur Johnson, Senior Scientific Analyst/Writer.

information on the composition of gum arabic and various Acacia species; UV spectral analyses on Acacia Senegal Gum and Acacia Concinna Fruit Extract; impurities analysis for pesticide residues in gum arabic; human skin tolerance test (skin irritation evaluated) on 2% Acacia Concinna Fruit Extract; and human maximization test data on a mascara containing 8% Acacia Senegal. Based on this new information, the Panel has prepared this amended safety assessment.

The terminology with which the cosmetics industry describes these ingredients has changed over the past several years. Table 1 shows the progression of terminology from the mid-1990s to 2004. In some cases (e.g., Acacia Concinna Fruit Extract, Acacia Dealbata Leaf Wax, and Acacia Farnesiana Gum) the current name for the ingredient (Gottschalck and McEwen 2004) better reflects the source of the plant material. The current terminology will be used in this report.

A key factor in the determination that the current data are sufficient was the finding that gum arabic is the equivalent of Acacia Senegal Gum. Accordingly, the following is background information on gum arabic and its relationship to gum produced by the *Acacia senegal* plant.

Sudan is the world's largest producer of gum arabic, and it is the main source of gum in international trade. Nigeria is the second largest producer of gum arabic. In the Sudan, the term gum arabic is inclusive of two types of gum that are produced and marketed, "hashab" (from *Acacia senegal*) and "talha" (from *Acacia seyal*). Gum arabic (hashab) from the Sudan is considered to be of the highest quality, and sets the standard by which other "gum arabics" are judged. *Acacia senegal* intrinsically produces a high-quality exudate (pale to orange-brown-colored solid) with superior technical performance; and, in the Sudan, the collection, cleaning, sorting, and handling of it up to the time of export is well organized and highly efficient. In a wider sense, the name gum arabic often is understood to mean the gum from any *Acacia* species and is sometimes referred to as "Acacia gum." For example, gum arabic from Zimbabwe is derived from *Acacia karroo* (Food and Agriculture Organization of the United Nations 1999). Acacia Senegal Gum has been described as the major commercial Acacia gum (Anderson 1988).

Although most internationally traded gum arabic comes from *Acacia senegal*, the term "gum arabic" may not imply a particular botanical source. In a few cases, so-called gum arabic may not even have been collected from Acacia species, but may originate from *Combretum*, *Albizia*, or some other genus (Food and Agriculture Organization of the United Nations 1999).

In the *International Cosmetic Ingredient Dictionary and Handbook*, gum arabic is listed as a technical name for Acacia Catechu Gum, Acacia Farnesiana Gum, and Acacia Senegal Gum (Gottschalck and McEwen 2004). However, since this publication, the Cosmetic, Toiletry, and Fragrance Association (CTFA) determined that gum arabic does not apply to Acacia Catechu Gum or Acacia Farnesiana Gum and will no longer be listed in the *International Cosmetic Ingredient Dictionary and Handbook* as a technical/other name for these ingredients (CTFA 2000b).

According to CTFA, gum arabic applies to the dried gummy exudate from branches and stems of *Acacia senegal* and other Acacia species from Africa, and *Acacia catechu* and *Acacia farnesiana* are not African species (CTFA 2000b).

This definition is similar to the following definition of Acacia that is found in the *National Formulary* (United States Pharmacopeial Convention 2000): Acacia is the dried gummy exudate from the stems and branches of *Acacia senegal* (Linné) Wildenow or of other related African species of Acacia (Family Leguminosae). It has also been described as a complex mixture of calcium, magnesium, and potassium salts of arabic acid. Arabic acid is a complex of galactose, rhamnose, arabinose, and glucuronic acid (Frutarom Meer Corporation, no date).

Gum arabic is a substance that is generally recognized as safe (GRAS) for direct addition to human food under the provisions of Section 184.1330 of the Code of Federal Regulations (21 CFR 184.1330). A report, prepared for the Food and Drug Administration (FDA), summarizing all available scientific data (1920 to 1972) related to the safety of gum arabic as a food ingredient has been published (Informatics Inc. 1972). Studies from that report are referenced in the text of this report.

In a subsequent report (prepared for FDA) evaluating the safety of gum arabic as a food ingredient, the Select Committee on GRAS Substances of the Life Sciences Research Office, Federation of American Societies for Experimental Biology (FASEB), concluded that "there is no evidence in the available information on gum arabic that demonstrates a hazard to the public when it is used at levels that are now current and in the manner now practiced. However, it is not possible to determine, without additional data, whether a significant increase in consumption would constitute a dietary hazard" (FASEB 1973).

The Select Committee also determined that additional experiments should be undertaken to evaluate the significance of gum Arabic allergenicity to the population as a whole, and that it may be advisable to conduct feeding studies in several animal species (including pregnant animals) at dosage levels that approximate and exceed the current maximum daily human intake (see "Noncosmetic Use").

Studies from the 1973 FASEB report are summarized in the text of this report. Studies on Acacia Senegal Gum and other species of Acacia (listed in the *International Cosmetic Ingredient Dictionary and Handbook* and those not listed) that have been published since the FASEB report was issued are also included. To ensure that the information in the present report is representative of the published chemistry and toxicity data on species of Acacia, the data presented involve various parts/components of the Acacia tree as well as the gummy exudate.

CHEMISTRY

Definitions of various ingredients derived from Acacia species in the *International Cosmetic Ingredient Dictionary and*

TABLE 1
Acacia-derived cosmetic ingredient terminology, description, and function

1995–1997 Terminology (Wenninger and McEwen 1995, 1997)			2004 Terminology (Gottschalck and McEwen 2004)		
Name	Description	Cosmetics function	Name	Description	Cosmetics function
Acacia Catechu	Plant material derived from *Acacia catechu*	Biological additive	Acacia Catechu	EU term for Acacia Catechu Gum	N/A
Acacia Catechu	Dried, crushed core of *Acacia catechu*	Biological additive	Acacia Catechu Gum	Dried, crushed core of *Acacia catechu*	Not reported
Acacia Concinna	Plant material derived from *Acacia concinna*	Not reported	Acacia Concinna	EU term for Acacia Concinna Fruit Extract	N/A
Acacia Concinna Extract	Extract of the fruit of *Acacia concinna*	Biological additive	Acacia Concinna Fruit Extract	Extract of the fruit of *Acacia concinna*	Not reported
Acacia Dealbata	Plant material derived from *Acacia dealbata*	Not reported	Acacia Dealbata	EU term for Acacia Dealbata Leaf Extract	N/A
Acacia Dealbata Extract	Extract of the leaves of *Acacia dealbata*	Biological additive	Acacia Dealbata Leaf Extract	Extract of the leaves of the wattle, *Acacia dealbata*	Not reported
			Acacia Dealbata Leaf Wax	Wax obtained from the leaves of *Acacia dealbata*	Skin-conditioning agent—emollient; skin protectant
Acacia Decurrens	Plant material derived from *Acacia decurrens*	Not reported	Acacia Decurrens	EU term for Acacia Decurrens Extract	N/A
Acacia Decurrens Extract	Extract of the acacia, *Acacia decurrens*	Biological additive	Acacia Decurrens Extract	Extract of the acacia, *Acacia decurrens*	Astringent; Skin-conditioning agent—Occlusive
Acacia Farnesiana	Plant material derived from *Acacia farnesiana*	Not reported	Acacia Farnesiana	EU term for Acacia Farnesiana Extract, Flower Wax, and Gum	N/A
Acacia Farnesiana	Plant material derived from the dried, gummy exudate of the acacia, *Acacia farnesiana*	Not reported	Acacia Farnesiana Gum	Plant material derived from the dried, gummy exudate of the acacia, *Acacia farnesiana*	Not reported
Acacia Farnesiana Extract	Extract of the flowers and stems of the the acacia, *Acacia farnesiana*	Biological additive	Acacia Farnesiana Extract	Extract of the flowers and stems of the the acacia, *Acacia farnesiana*	Astringent
			Acacia Farnesiana Flower Wax	wax obtained from the flowers of *Acacia farnesiana*	Skin protectant
Acacia Senegal	Plant material derived from *Acacia senegal*	Not reported	Acacia Senegal	EU term for Acacia Senegal Extract, Gum, and Gum Extract	N/A

(Continued on next page)

TABLE 1
Acacia-derived cosmetic ingredient terminology, description, and function *(Continued)*

1995–1997 Terminology (Wenninger and McEwen 1995, 1997)			2004 Terminology (Gottschalck and McEwen 2004)		
Name	Description	Cosmetics function	Name	Description	Cosmetics function
Acacia senegal	Plant material derived from the dried, gummy exudate of the acacia, *Acacia senegal*	Not reported	Acacia Senegal Gum	Plant material derived from the dried, gummy exudate of the acacia, *Acacia senegal*	Not reported
Acacia Senegal Extract	Extract of the flowers and stems of the the acacia, *Acacia senegal*	Biological additive	Acacia Senegal Extract	Extract of the flowers and stems of the acacia, *Acacia senegal*	Not reported
Acacia Senegal Gum Extract	Extract of the gum of the acacia, *Acacia senegal*	Biological additive	Acacia Senegal Gum Extract	Extract of the gum of the acacia, *Acacia senegal*	Not reported

Handbook are included in Table 1 (Gottschalck and McEwen 2004). CAS numbers are listed for the following two: Acacia Catechu Gum (CAS no. 8001-76-1) and Acacia Senegal Gum (CAS no. 9000-01-5).

Chemical and Physical Properties

Gum Arabic

The gummy exudate from the *Acacia senegal* is a proteinaceous polysaccharide, with protein content ranging from approximately 1.5% to 3% for samples from various producing areas (World Health Organization 1990).

Gum arabic is a white powder that is readily soluble in water, but insoluble in alcohol (Anonymous 1993). Molecular weights of ~850,000 (Ross et al. 1984a, 1984b) and ~240,000 (Frutarom Meer Corporation no date), and a density of 1.35 to 1.49 (Dangerous Properties of Industrial Materials Report 1981) have been reported. An aqueous solution is acid to litmus (Lewis 1993a).

Pazur et al. (1986) indicated that gum arabic is composed of D-galactose, L-rhamnose, L-arabinose, and D-glucuronic acid residues in an arrangement of a main chain of galactosyl units joined by β-D-(1 $\rightarrow$ 3) linkages and side chains or branched oligosaccharides linked to the main chain by β-D-(1 $\rightarrow$ 6) linkages. The oligosaccharides may contain terminal rhamnosyl units linked (1 $\rightarrow$ 3) or terminal arabinofuranosyl units linked (1 $\rightarrow$ 4) to internal galactosyl or glucuronosyl units. Based on methylation and degradation studies of gum arabic (*Acacia senegal*) along with periodate oxidation and other confirmatory reactions, the structure for gum arabic shown in Figure 1 was proposed (Informatics Inc. 1972).

Gum arabic is almost completely soluble in twice its weight of cold water, and the viscosity of the gum increases slowly at concentrations up to 25%. At concentrations greater than 25%, the viscosity increases much more rapidly in proportion to the gum content (Frutarom Meer Corporation no date).

UV Absorption

An increase in absorbance for Acacia Senegal was observed between 400 nm and approximately 260 nm, reaching a plateau at wavelengths ranging from 270 to ~250 nm. A rapid increase in absorbance was observed at wavelengths less than 250 nm (Avon Products, Inc. 2000a). UV absorption spectra provided on two other lots of Acacia gum (Acacia Senegal) were both similar to the preceding UV spectral analysis (Avon Products, Inc. 2000b).

Methods of Production

Gum arabic is produced when the Acacia tree is stressed by infection, poor nutrition, heat, or lack of moisture. The gum exudes through wounds in the bark that occur naturally or are purposely made to stimulate production. The exudate dries rapidly, is collected as hardened drops or tears, sorted, graded, and marketed. The gum becomes harder during storage; market preferences exist for both the harder (old) and softer (new) gum (FASEB 1973).

According to another source, the removal of the bark that adheres to the tears is critical to the production of quality gum arabic. Additionally, in order to produce quality products, elaborate processes for the preclearing of milled gum and the centrifugation and filtration of feed solutions for spray dried gum must be followed. The major growing regions for gum arabic are in the Sudan and West Africa, and the Kordofan grade is considered the best (Frutarom Meer Corporation, no date).

Gum arabic in solid form is imported from the Sudan. According to one source, the solid is converted to a liquid form and

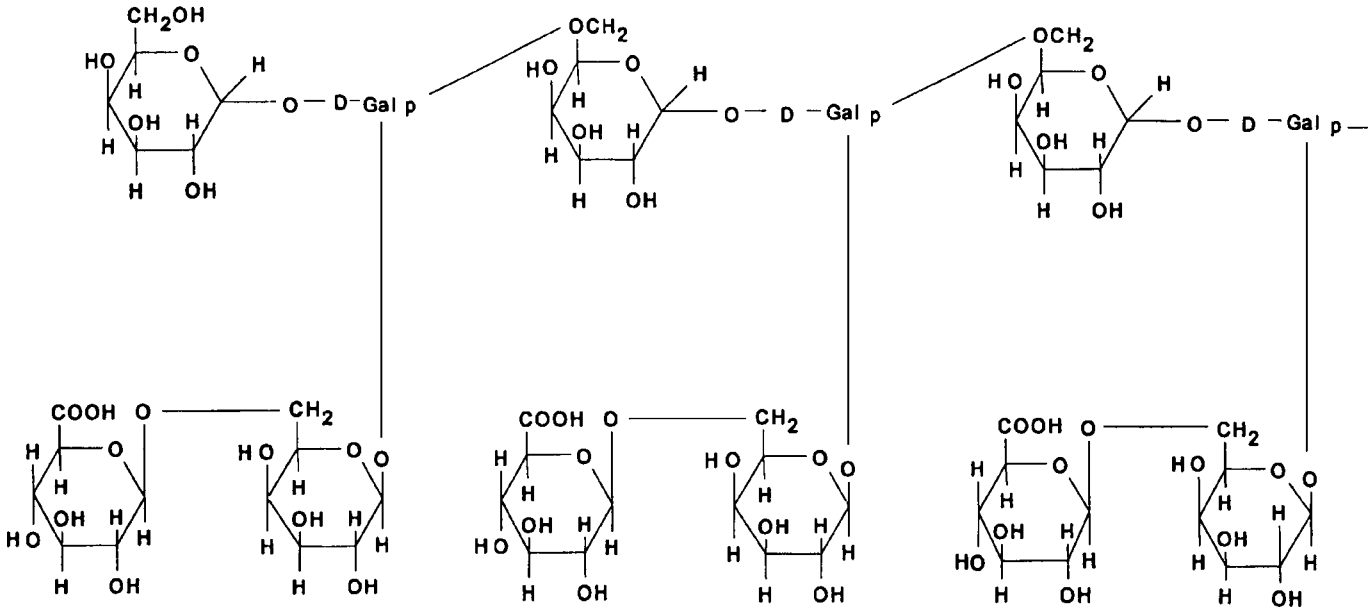

FIGURE 1
Proposed structure for Gum Arabic (Informatics 1972).

the preservatives Proxel GXL (0.13%) and sodium benzoate are then added. Proxel GXL consists of 20% 1,2-benzisothiazolin-3-one (BIT) in aqueous dipropylene glycol (Freeman 1984).

Crude Acacia Concinna results from the drying and pulverization of the pods of *Acacia concinna*. The extract of these pods (Acacia Concinna Fruit Extract) is drawn by cold processing (Carlisle International Corporation 1997a).

Composition, Analytical Methods, and Impurities

The following three grades of gum arabic have been noted in the published literature: (1) processed gum arabic recovered by spray-drying from a solution of commercial food grade gum arabic after filtration to remove sand, etc., and after heat treatment to effect pasteurization; (2) finely powdered natural gum arabic of poor commercial quality, giving solutions of a dark reddish brown color; (3) finely powdered natural gum arabic of very high quality, giving essentially colorless solutions (Strobel et al. 1982). Gum arabic has been analyzed by gas chromatography (Lawrence and Iyengar 1985) and has been identified by microelectrophoresis (Informatics Inc. 1972).

Powdered gum arabic contains moisture (15% maximum) and insoluble ash (0.5% maximum). The pH of a 10% solution is approximately 4.6 (Frutarom Meer Corporation, no date). The following specifications exist for United States Pharmacopoeia (USP) grade Acacia: loss on drying (15% maximum), total ash (4% maximum), arsenic (3 ppm), lead (0.001%), and heavy metals (0.004%) (United States Pharmacopeial Convention, Inc. 2000).

FDA has listed Acacia (Gum Arabic) as a direct food additive that meets the specifications of the *Food Chemicals Codex* (21 CFR 184.1330). The specifications for food grade Acacia include arsenic (3 mg/kg maximum); ash, acid-insoluble (0.5% maximum); ash, total (4% maximum); heavy metals (0.002% maximum); insoluble matter (1% maximum); lead (5 mg/kg maximum); and loss on drying (15% maximum) (Food Chemicals Codex 1996).

Anderson et al. (1990) compared the amino acid composition of Sudanese and Nigerian gum arabic. Analyses were done on samples collected over a 13-year period for samples from the Sudan and over 9 years for those from Nigeria. The data are presented in Table 2.

Anderson et al. (1991) analyzed gum arabic samples provided by importers shown in Table 3. All samples conformed to the revised Joint Food and Agriculture Organization of the United Nations/World Health Organization, Expert Committee on Food Additives (JECFA) specification in respect of solubility, complete in cold water; acid-insoluble ash, (>0.5%) and matter (>1%); starch/dextrin (absent); tannin (absent); arsenic (>3 ppm), lead (>10 ppm), heavy metals (>40 ppm) (JECFA 1990). All samples were confirmed by nuclear magnetic resonance (NMR) spectroscopy to be "good" *Acacia senegal*.

Anderson et al. (1990) also analyzed the ash from gum arabic samples from these same two countries. These data are given in Table 4.

West Coast Analytical Service, Inc. (1999) analyzed gum arabic for pesticide residues using USP methodology and found no detectable pesticide residues.

Other food additive specifications for gum arabic from the Food and Agriculture Organization (FAO) of the United Nations defined the material as a dried exudate obtained from the stems and branches of *Acacia senegal* (L.) Willdenow or *Acacia seyal*

TABLE 2
The amino acid composition of Sudanese and Nigerian Gum Arabic (*Acacia senegal*) (Anderson et al. 1990)

Amino acid	Sudanese samples Mean residues/1000 residues (13 years total between 1904 and 1989)	Nigerian samples Mean residues/1000 residues (9 years total between 1905 and 1967)
Alanine	27 ± 3	24 ± 4
Arginine	13 ± 4	12 ± 1
Aspartic acid	68 ± 13	61 ± 16
Cystine	2 ± 4	0
Glutamic Acid	42 ± 10	42 ± 15
Glycine	50 ± 5	50 ± 6
Histidine	44 ± 8	48 ± 5
Hydroxyproline	304 ± 47	331 ± 73
Isoleucine	12 ± 3	13 ± 3
Leucine	66 ± 7	69 ± 8
Lysine	25 ± 3	24 ± 6
Methionine	2 ± 2	1
Phenylalanine	33 ± 5	29 ± 10
Proline	63 ± 14	55 ± 9
Serine	129 ± 11	129 ± 13
Threonine	68 ± 9	67 ± 8
Tyrosine	14 ± 5	14 ± 4
Valine	35 ± 8	32 ± 6

(family Leguminosae), include the following: loss on drying (not more than 15% [105°C, 5 h] for granular and not more than 10% [105°C, 4 h] for spray dried material); total ash (not more than 4%), acid-insoluble ash (not more than 0.5%), acid-insoluble matter (not more than 1%); and lead (not more than 2 mg/kg) (FAO 1999).

TABLE 4
Cation composition of ash from Sudanese and Nigerian Gum Arabic (*Acacia senegal*) (Anderson et al. 1990)

Cation	Sudanese samples Mean μg/g ash unless expressed as ppm (15 years total between 1904 and 1989)	Nigerian samples Mean μg/g ash unless expressed as ppm (9 years total between 1905 and 1967)
Aluminum	190 ± 53	311 ± 156[a]
Calcium	256,000 ± 34,000	316,000 ± 56,000
Chromium	47 ± 22	34 ± 26
Copper	52 ± 27	66 ± 65[b]
Iron	128 ± 84	110 ± 33
Lead	6 ± 2	11 ± 7
Magnesium	38,000 ± 15,000	39,000 ± 15,000
Manganese	100 ± 95	57 ± 27
Nickel	10 ± 11	12 ± 17
Potassium	237,000 ± 37,000	221,000 ± 43,000
Sodium	9,400 ± 4,480	10,200 ± 5,200
Zinc	24 ± 10	40 ± 49[c]
Arsenic	<1 ppm	<1 ppm
Cadmium	<1 ppm	<1 ppm
Cobalt	<1 ppm	<1 ppm
Molybdenum	<1 ppm	<1 ppm

[a]Mean = 266 ($n = 8$) if one value, 675, is treated as an outlier.
[b]Mean = 47, if one value, 225, is treated as an outlier.
[c]Mean = 25, if one value, 159, is treated as an outlier.

Acacia Concinna Fruit Extract

Acacia Concinna Fruit Extract consists of 1 part of extract obtained from 1 part of dry pods of *Acacia concinna*. It contains the active constituents of the pods of *Acacia concinna*, such as

TABLE 3
Analytical data for natural Gum Arabic samples provided by importers in 1990/1991 (Anderson et al. 1991)

Sample no.	Sample sent by	Date received	% H_2O[a]	% Ash[a]	% N[a]	Specific rotation (degrees)
N1	American importer A	12/90	13.2	3.8	0.34	−29
N2	American importer A	12/90	13.9	4.0	0.36	−31
N3	Italian importer B	12/90	14.4	3.3	0.31	−30
N4	British importer C	12/90	14.5	3.6	0.37	−31
N5	British importer D	12/90	12.2	3.5	0.35	−33
N6	British importer E	12/90	14.9	2.0	0.38	−33
N7	German importer A	1/91	14.4	4.0	0.26	−34
N8	American importer G	1/91	15.0	3.2	0.29	−26
N9	American importer H	1/91	13.9	3.9	0.33	−32
N10	British importer K	2/91	13.3	3.7	0.34	−28
N11	Italian importer L	2/91	14.8	3.4	0.30	−29
Mean values			14.0	3.6	0.33	−30.5

[a]Dry-weight basis, as specified (Food and Agriculture Organization of the United Nations 1990).

TABLE 5
Acacia Concinna Fruit Extract specifications (Carlisle International Corporation 1997a)

Specification	Standard
Color	Brown
pH	4 to 6
Specific gravity (at 25°C)	1.0 to 1.10
Refractive index (at 20°C)	1.1 to 1.4
Dried residue (2 h/110°C)	10% to 20%
Water	60% to 65%
Propylene glycol	35% to 40%
Water solubility	Soluble
Preservatives	Parabens and potassium sorbate
Heavy metal	<10 ppm
UV/VIS spectrophotometry	
Absorbance at 220 nm of a 0.1% aqueous solution	1.0 ± 0.25
Absorbance at 220 nm of a 0.2% aqueous solution	2.0 ± 0.20
Other constituents (HPTLC method)	Saponins, alkaloids, malic acid
Maximum total bacterial count	100/g
Maximum yeasts and molds	0/g

vegetable saponins. The raw material (*Acacia concinna*) from which Acacia Concinna Fruit Extract is derived is from wild, crafted sources free of contamination with pesticide residues. The standard analytical profile of Acacia Concinna Fruit Extract is given in Table 5. A sample "passes" if it meets these specifications (Carlisle International Corporation 1997a).

Information on the composition and impurities of various species of Acacia and their contaminants is included in Table 6. The Acacia species that are listed in the *International Cosmetic Ingredient Dictionary and Handbook* are identified with an asterisk.

As noted in Table 6, aflatoxin has been detected in the bark and seeds of *Acacia catechu* (Roy and Kumari 1988, 1991). Abdalla (1988) also described gum-yielding Acacia twigs from the Sudan (supplier of Acacia Senegal Gum) as a source of aflatoxin (81 to >1000 μg/kg).

Smith et al. (1990), however, found no detectable aflatoxin in either of two samples of gum arabic analyzed using an enzyme-linked immunosorbent assay. The assay system was capable of determining aflatoxin in the concentration range of 2.0 to 200.0 ppb in gum arabic.

Data from the European Federation for Cosmetic Ingredients (EF*f*CI) describes the components of the plant material from various Acacia species. While there are some similarities, there are many differences in composition (EF*f*CI 2000). These data are given in Table 7.

Reactivity

When Gum Acacia is weakly hydrolyzed by hydrochloric acid at room temperature, pentose is split off (Marrack and Carpenter 1938). Partial acid hydrolysis has also yielded galactose and complex sugar acids (Heidelberger et al. 1929).

Gum Acacia emits acrid smoke when heated to decomposition (Lewis 1993b). Heating a solution of Acacia for a few minutes at 100°C destroys peroxidase (oxidizing agent) present in the gum and the colored derivatives produced (Gennaro 1990).

USE

Purpose in Cosmetics

The functions of these ingredients in cosmetics as described in the *International Cosmetic Ingredient Dictionary and Handbook* are given in Table 1 (Wenninger et al. 2000).

Reportedly, *Acacia concinna* pods are a useful hair wash, in that they promote hair growth, kill lice, and remove dandruff. The active constituents of *Acacia concinna* pods (saponins, alkaloids, tannins, and malic acid) are said to have cleansing, stimulating, and astringent properties. The astringent action provides toning of the scalp and conditioning of the hair. Additionally, the active constituents are said to offer effective skin and scalp exfoliation (Carlisle International Corporation 1997b).

Scope and Extent of Use in Cosmetics

The product formulation data submitted to the FDA in 2001 indicated that Acacia was used in 33 cosmetic products and that Acacia Senegal was used in 1 cosmetic product (Table 8) (FDA 2001). Neither the species nor the plant part was further delineated in the category "Acacia." It is assumed that Acacia Senegal is Acacia Senegal Gum.

Current concentration of use data are given in Table 8. These data from industry (CTFA 2000a) show the highest concentration of Acacia Senegal Gum (9%) in shampoos. Acacia Senegal Gum Extract was reported at a concentration of 0.001% in bath soaps and detergents. For many uses of these ingredients, information regarding use concentration for specific product categories is provided, but the number of such products is not known, but they must be assumed to be in use.

Recommended use concentrations of Acacia Concinna Fruit Extract are 0.5% to 5.0% (Carlisle International Corporation 1997a) and 1.0% to 2.0% for use in shampoos, hair packs, hair conditioners, and hair rinses (Carlisle International Corporation 1997b).

Cosmetic products containing Acacia are applied to most parts of the body and could come in contact with the ocular and nasal mucosae. These products could be used on a daily basis, and could be applied frequently over a period of several years.

Acacias are not included among the substances listed as prohibited from use in cosmetic products that are marketed in the European Union (EEC 2001). The European Union terminology

TABLE 6
Composition and impurities data on Various Species of Acacia

Acacia species (part/source)	Analytical method	Components	Reference
Acacia atramentaria and *Acacia tortuosa* (leaves)	Gas chromatography and NMR spectroscopy	Proacacipetalin (cyanogenic glucoside)	Seigler et al. 1983
*Acacia albida, Acacia ataxa-cantha, Acacia catechu**, *Acacia confusa, Acacia coulteri, Acacia erubescens, Acacia ferruginea, Acacia galpinii, Acacia hamulosa, Acacia mellifera, Acacia modesta, Acacia nigrescens, Acacia polyacantha, Acacia rovumae, Acacia senegal**, *Acacia venosa*, and *Acacia welwitschii* (seeds)	Ion exchange chromatography	α-Amino-β-oxalylaminopropionic acid (neurotoxic lathyrogen)	Quereshi et al. 1977
Acacia aroma (leaves)	Gas chromatography and NMR spectroscopy	Linamarin and lotaustralin (cyanogenic glucosides)	Seigler et al. 1983
*Acacia catechu** (seed)	Thin-layer chromatography and spectrophotometry	Aflatoxin B_1 (0.01 to 0.76 μg/g)	Roy and Kumari, 1991
*Acacia concinna** (pods)	—	Highly polar saponin mixture. Hydrolysis with alkali yields 5 triterpenoidal prosapogenols (concinnosides A, B, C, and D), 4 glycosides (acadiaside, julibroside A1, julibroside A3, albiziasaponin C), and aglycone, acacic acid lactone	Abul et al. 1997
*Acacia concinna** (fruit)	—	Kinmoonsides A–C (3 cytotoxic saponins)	Tezuka et al. 2000
*Acacia farnesiana** (pod, leaf, stem, old stem, and flower)	Phytochemical screening	Carbohydrates and/or glycosides, reducing sugars, hydrolyzable tannins, alkaloids and nitrogenous bases, unsaturated sterols, and/or terpenes, and coumarins (all organs)	Wassel et al. 1992
*Acacia farnesiana** (pod, leaf, old stem, and flower)	—	Flavonoids (all organs except stem)	Wassel et al. 1992
*Acacia farnesiana** (pod, leaf, stem)	—	Cyanogenic glycosides (in pod, leaf, and stem)	Wassel et al. 1992
*Acacia farnesiana** (flower)	—	Volatiles (flower)	Wassel et al. 1992
*Acacia farnesiana** oil	Thin layer chromatography	Anisaldehyde, benzalcohol, benzaldehyde, cuminicalcohol, farnesol, cuminicaldehyde, geraniol, geranyl acetate, ionone, linalool, linalyl acetate, nerolidol, terpineol, and methyl salicylate	El-Hamid and Sidrak 1970
*Acacia farnesiana** (leaves)	—	Total soluble phenols ranged from 10.27% to 35.46%. Condensed tannins ranged from 0.5% to 8.28% on dry matter basis	Sotohy et al. 1995
*Acacia farnesiana** (leaves)	—	Cyanogenic glycoside (linamarin or lotaustralin may be present)	Secor et al. 1976

(Continued on next page)

TABLE 6
Composition and impurities data on Various Species of Acacia *(Continued)*

Acacia species (part/source)	Analytical method	Components	Reference
Acacia tortilis (gum and bark extracts)	High-performance liquid chromatography	Smooth muscle relaxants: quaracol A and B (in gum) and (+)-fisetinidol (in gum and bark)	Hagos and Samuelson 1988
Acacia georginae (seeds)	Extractive and chromatographic procedures	Fluoroacetic acid	Oelrichs and McEwan 1962
Acacia globulifera (leaves)	Gas chromatography and NMR spectroscopy	Epiproacacipetalin (cyanogenic glucoside)	Seigler et al. 1983
Acacia modesta (stem bark, heartwood, and leaf extracts)	Thin-layer chromatography	α-amyrin, betulin, octacosanol and ε-sitosterol (in stem bark); γ-sitosterol and pinitol (in heartwood); octacosane, hentriacontane, octacosanol, and hentriacontanol (leaves)	Joshi et al. 1975
*Acacia mollissima, Acacia confusa, Acacia longifolia, Acacia decur-rence**, *Acacia dealbata**, *Acacia baileyana*, and *Acacia verticillata* (leaves)	Amino acid autoanalyzer used	(−)-*trans*-4-hydroxypipecolic acid	Marakesh et al. 1969

*The Acacia species listed in the *International Cosmetic Ingredient Dictionary.*

for these ingredients is described in Table 1, where the genus and species names are used to describe all of the plant material (e.g., gum, extract, etc.) derived from that particular genus/species, independent of the plant part from which the material is derived.

The Acacias reviewed in this report are not included on the list of ingredients that must not be combined in cosmetic products that are marketed in Japan (Ministry of Health, Labor and Welfare [MHLW] 2000a) or on the list of restricted ingredients for cosmetic products that are marketed in Japan (MHLW 2000b).

Noncosmetic Use

Gum arabic is a substance that is generally recognized as safe (GRAS) for direct addition to human food under the provisions of Section 184.1330 of the Code of Federal Regulations (CFR). It is approved for use in various food categories at the following maximum permitted usage levels: 2.0% (beverage and beverage bases), 5.6% (chewing gum), 12.4% (confections and frostings), 1.3% (dairy product analogs), 1.5% (fats and oils), 2.5% (gelatins, puddings, and fillings), 46.5% (hard candy and cough drops), 8.3% (nuts and nut products), 6.0% (quiescently frozen confection products), 4.0% (snack foods), 85.0% (soft candy), and 1% (all other food categories).

Uses of gum arabic in the various food categories include: emulsifier and emulsifier salt, flavoring agent and adjuvant, formulation aid, stabilizer and thickener, humectant, surface-finishing agent, processing aid, and texturizer (21 CFR 184.1330). Gum arabic is also listed as one of the optional blending ingredients of vanilla powder (21 CFR 169.179) and vanilla-vanillin powder (21 CFR 169.182).

The following maximum values for possible daily human intake (g/kg body weight) of gum arabic in the total diet have been calculated for various age groups by the Select Committee on GRAS Substances using data from the National Research Council: 115 mg/kg (0 to 5 months), 322 mg/kg (6 to 11 months), 329 mg/kg (12 to 23 months), and 113 mg/kg (2 to 65 + years) (FASEB 1973).

At the 35th meeting of the JECFA, held in Rome from May 29 to June 7, 1989, JECFA confirmed its acceptable daily intake (ADI) of gum arabic as "not specified." Here, gum arabic (a.k.a. gum Acacia) is defined as the dried gummy exudate from tropical and subtropical *Acacia senegal* trees.

ADI "not specified" is applicable to a food substance of very low toxicity, which, on the basis of the available data (chemical, biochemical, toxicological, and other), the total dietary intake of the substance arising from its use at the levels necessary to achieve the desired effect, and from its acceptable background in food does not, in the opinion of the JECFA, represent a hazard to health. For that reason, and for reasons stated in individual evaluations, the establishment of an ADI expressed in numerical form is not deemed necessary. An additive meeting this criterion must be used within the bounds of good manufacturing practice, i.e., it should be technologically efficacious and should be used at the lowest level necessary to achieve this effect; it should not conceal inferior food quality or adulteration, and it should not create a nutritional imbalance (JECFA 1990).

TABLE 7
Chemicals found in Acacia species (EF*f*CI 2000)

Acacia catechu plant	Acacia decurrens plant	Acacia farnesiana plant	Acacia senegal plant
(+)-afzelichin	(+)-catechin	(+)-catechol	4-methoxyglucuronic acid
3-(beta-L-arabopyranoside)-L-arabinose	3,3′,4′,5′,7-pentahydroxy-2-phenylchroman	(+)-gallocatechol	Arabic acid
3-methoxyflavones	3,3′,4,4′,7′-pentahydroxyflavin	Apigenin-6,8-bis-beta-D-glucopyranoside	Arabinose
4-(4-*O*-methyl-alpha-D-glucuronoside)-L-arabinose	3-methyl-L-rhamnose	Aromadendrin	Ascorbic acid
4-hydroxypipecolic acid	7,3′,4′,5-tetrahydroxyflavan-3-O-L-catechin	Aspartic acid	Aspartic acid
5-(beta-D-xylopyranoside)-L-arabinose	Acetic acid	Cresols	Beta sitosterol
7,3,4-trihydroxy-3,8-dimethoxyflavone	Aldobionic acid	Ellagic acid	Beta sitosterol-D-glucose
7,8,14′-trihydroxyflavonol	Alpha cellulose	Ethyl ester	Cysteine
7,8,4-trihydroxy-3-methoxyflavone	Anthocyanidin	Hydroxyacetophenone	D-galactose
8-methoxyfisetin	Anthocyanilidine	Isorhamnetin-3-rutinoside	D-glucoside
9-methoxyflavone-3,4-diones	Beta carotene	Kaempferol	Dimethyltryptamine
Acacatechin	Carbohydrates	Kaempferol-7-galloylglucose	Erythrodiol
Acetaldehyde	Cellulose	Kaempferol-7-glucoside	Galactoglucuronid acid
Aldobiuronic acid	D-galacturonic acid	Linamarin	Glucuronic acid
Alpha-amino-beta-oxalylaminopropionic acid	D-pinetol	Lotaustralin	HCN
Alpha catechin	Fiber	*m*-digallic acid	Hentriacontane
Beta catechin	Fisetinidin	Methyl gallate	Hentriacontanol
Boron	Fructose	Mucilage	Kaempferol
Catechuic acid	Gallocatechin	Myricetin-4′-methylether-3-rhamnoside	L-arabinose
Catechutannic acid	Indoleacetic acid	*N*-acetyl-djenkkolic acid	L-rhamnose
Cobalt	L-arabinose	Naringenin-7-glucoside	Leucine
D-galactose	L-rhamnose	Naringenin-7-rhamnoglucoside	Magnesium
D-glucuronic acid	Lignin	Pipecholic acid	Octacosanol
D-xylose	Mearnsitrin	Prunin-*O*-6′-gallate	Peroxidase
Diamino acid	Methanol	Quercetin-3-*O*-rutinoside	Potassium
Dihydrokaempferol	Methylsalicylic ester	Salicylic acid	Quercetin
DL-catechol	Pelargonidin	Tyramine	Rhamnose
DL-epicatechin	Phlobaphene		Rhamnose hydrate
Fisetin	Phlobaphene anhydride		Serine
Flavotannin	Phloroglucinol		Sitosterol
Formaldehyde	Proanthocyanidin		Sodium

(Continued on next page)

TABLE 7
Chemicals found in Acacia species (EF*f*CI, 2000) *(Continued)*

Acacia catechu plant	Acacia decurrens plant	Acacia farnesiana plant	Acacia senegal plant
Gallic acid	Protocatechuic acid		Sucrose
Gallotannin	Robinetin		Tannin
Gamma-catechin	Rutin		Uronic acid
Glucosyluronic acid	Xanthophyll		Valine
Gum			
Isocacatechin			
Isorhamnetin			
Isovaleraldehyde			
Kaempferol			
L-epicatechin			
L-leucomacluricglycol ether			
L-rhamnose			
Magnesium			
Malate dehydrogenase			
Manganese			
Peroxidase			
Phlobatannin			
Phosphatase			
Procyanidin			
Quercetagetin			
Quercetin			
Quercitrin			
Rutin			
Silicon			
Tannin			
Taxifolin			
Uronic acid			

Gum arabic (Acacia Senegal Gum) is used in the pharmaceutical industry to stabilize emulsions during the preparation of tablets (Collins et al. 1987). It is also used for its demulcent action in the treatment of throat or gastric inflammation (Gennaro 1990).

The therapeutic efficacy of Acacia Catechu in the treatment of lepromatous leprosy has been reported (Ojha et al. 1969).

Gum Arabic has also been used in glues, lithographic solutions, and matches (tip and binder in striking surface), and polisher and textile finishes (van Ketel 1984).

The following uses of Acacia Concinna in folk medicine have been reported: A chutney (pungent relish of fruits, spices, and herbs) made of the tender leaves of *Acacia concinna*, salt tamarind, and chilies is administered for the treatment of bilious affections such as jaundice. An infusion of the leaves is used in the treatment of malarial fever; it checks flatulence and serves as a mild laxative. Furthermore, repeated, large doses of a decoction of the *Acacia concinna* pods act as an emetic and purgative (Carlisle International Corporation 1997b).

An ointment made from the *Acacia concinna* pods reportedly is used in the treatment of skin diseases (Carlisle International Corporation 1997b).

BIOLOGICAL PROPERTIES

Absorption, Distribution, Metabolism, and Excretion

Gum Arabic

The weight gain for rats fed gum arabic at a dietary concentration of 16% was 75% of that reported for control rats. It was determined that approximately 80% of the gum arabic was absorbed (Informatics 1972).

In a study using rats, an apparent decrease in the caloric value of gum arabic with increasing administered dose was noted. Gum arabic was incorporated into the diet at concentrations of 5%, 10%, and 17%. Digestibility data indicated that up to 80% of the gum arabic was absorbed (Informatics Inc. 1972).

Following a 48 h fast, 20 young male rats were fed 10 mg of a mixture consisting of 34% white, powdered gum arabic and 66% cacao butter. At 72 h after feeding, the rats were anesthetized

TABLE 8
Product formulation data on Acacia and Acacia Senegal

Product category (total formulations in category) (FDA 2001)	Formulations with ingredient (FDA 2001)	Concentration of use (CTFA 2000a) (%)
Acacia		
Other bath preparations (193)	1	—
Mascara (187)	18	—
Other eye makeup preparations (151)	2	—
Hair tints (49)	1	—
Hair color sprays (Aerosol) (5)	1	—
Other hair-coloring preparations (59)	3	—
Foundations (319)	1	—
Lipstick (942)	1	—
Other makeup preparations (186)	1	—
Body and hand skin care preparations (excluding shaving) (827)	3	—
Paste masks (mud packs) (269)	1	—
2001 totals for Acacia	33	—
Acacia Senegal Gum		
Eyebrow pencil	—	1
Eyeliner	—	3
Mascara (187)	1	3–9
Powders (dusting and talcum; excluding aftershave talc)	—	0.5
Tonics, dressings, and other hair grooming aids	—	0.0001
Other skin care preparations	—	0.02
2001 totals for Acacia Senegal Gum	1	—
Acacia Senegal Gum Extract		
Bath soaps and detergents	—	0.001
2001 totals for Acacia Senegal Gum Extract	—	—

and the liver was removed and analyzed for glycogen content. The difference in glycogen concentration between control and fed rats was insignificant. Therefore, it was concluded that the gum arabic molecule was not metabolized by enzymes of the rat digestive tract (Informatics Inc. 1972; FASEB 1973).

Other studies have indicated that gum arabic is partially digested in the rat. In one study, weight gain and feed efficiency were determined using groups of six rats fed 15% gum arabic for 62 days. Feed efficiency was identical between experimental and control groups. However, compared to the control group (mean weight gain = 199 g), rats fed gum arabic had a mean weight gain of 224 g. In another study, groups of five rats were pair-fed gum arabic (0.75 g/day; added to 5 g basal diet). Results indicated that the digestibility of gum arabic was 71% (Informatics Inc. 1972).

Ross et al. (1984b) evaluated the metabolism of gum arabic using albino Wistar male rats (3 months old; weights = 350 g). The number of animals used in the study was not stated. Two groups of animals were fed Oxoid breeders diet only and Oxoid breeders diet plus 200 g gum arabic/kg ad libitum, respectively, for 4 weeks. Oxoid breeders diet was described as a reconstituted diet that allowed the ready incorporation of gum arabic into pellet form.

Feces were collected during the 24 h period before animals were killed. Following ad libitum overnight feeding, the animals were killed using a combination of diethyl ether anesthesia and cervical dislocation and contents from the stomach, small bowel, cecum, and distal colon were removed.

For rats fed gum arabic in the diet, a white flocculent precipitate typical of gum arabic was detected in contents from the stomach and small intestine, but not from the cecum, distal colon, or in the feces. The fact that precipitable gum arabic was detected along the gastrointestinal (GI) tract as far as the terminal ileum, but not in the cecum, suggests that the metabolism of gum arabic is mediated by bacteria in the cecum.

In animals in which the cecum was resected, precipitable gum arabic was detected along the length of the entire residual intestine. This observation suggests that in the absence of the bacterial mass resident in the cecum, there is no degradation of gum arabic. No precipitate typical of gum arabic was found in

the GI tract of control rats that received the Oxoid breeders diet only (Ross et al. 1984b).

A total caloric intake slightly greater than that for starch has been reported for gum arabic in rabbits. Evidence of glycogenesis was also demonstrated in this study. Thus, it appears that rabbits are able to utilize gum arabic (FASEB 1973).

In a study involving guinea pigs, it was determined that gum arabic was highly digestible (90%) when administered in the diet at a concentration of 15% for 10 days (Informatics 1972).

Results of studies in which dogs and rabbits were injected intravenously with gum arabic indicated that gum arabic or some other product associated with it accumulated in the liver and remained in the tissues for several months. Nonlethal effects included serious disturbances in hemoglobin, white blood cells, and serum proteins (FASEB 1973).

Using many of the studies summarized above, the Select Committee on GRAS Substances determined in 1973 that gum arabic can be digested to simple sugars. However, it was also determined that conclusive evidence indicating that the intact gum arabic molecule is absorbed under normal conditions was lacking (FASEB 1973). It should also be noted that data on the fate of undigested gum arabic in male rats (Ross et al. 1984b) have been published since the FASEB report was issued. The results of this previously summarized study suggest that the bacterial mass resident in the cecum is responsible for the metabolism of gum arabic.

Hypotensive Activity

Acacia (Not Gum Arabic)

Sham et al. (1984) evaluated the hypotensive activity of *Acacia catechu* (aqueous extract of branches) using four groups of four anesthetized dogs (males and females; weights = 8 to 12 kg). The right femoral artery and vein were cannulated for blood pressure recordings and intravenous injection. After a 30-min equilibration period, *Acacia catechu* was injected (bolus injection) into dogs from each of the four groups. Doses ranged from <1 to ~ 2 mg/kg. Changes in mean arterial blood pressure (MAP) were recognized as differences between the steady MAP before injection and the lowest MAP after injection.

The results were presented as a log dose-response curve. *Acacia catechu* induced dose-related hypotensive responses. At high doses, the hypotensive effect lasted approximately 30 min. Based on experimentation with various blocking agents, it was determined that this effect was not mediated through α- and β-adrenergic, cholinergic, or histaminergic receptors, or related to autonomic ganglion transmission.

The hypotensive activity of *Acacia catechu* (aqueous extract of branches) was also evaluated using four groups of five male Sprague-Dawley rats (weights between 170 and 250 g) according to the procedure in the preceding paragraph; however, in this experiment, the left carotid artery and jugular vein were cannulated.

Acacia catechu induced dose-related hypotensive responses in rats over the range of doses tested (1 to 2 mg/kg). It was also determined that the hypotensive responses were not mediated through α- and β-adrenergic, cholinergic, or histaminergic receptors, or related to autonomic ganglion transmission (Sham et al. 1984).

These same authors reported that, in an in vitro experiment, *Acacia catechu* induced a dose-dependent relaxation of helical strips of rat tail artery that had been preconstricted with the vasoconstrictors arginine vasopressin and methoxamine, respectively. In the presence of arginine vasopressin, *Acacia catechu* was tested at concentrations of 0.01, 0.03, and 0.1 mg/ml. *Acacia catechu* was tested at concentrations of 0.1, 0.3, and 1 mg/ml in the presence of methoxamine (Sham et al. 1984).

Hypocholesterolemic Activity

Acacia (Not Gum Arabic)

Chaudhari and Hatwalne (1973) determined the hypocholesterolemic activity of the dried water extract of *Acacia catechu*, also known as katha in India. They used three groups of 10 male albino rats (weights = 100 to 125 g). One group was fed stock diet thoroughly mixed with 1% cholesterol, and a second group was fed stock diet thoroughly mixed with 1% cholesterol plus 0.2% katha. The control group was fed stock diet only. The diets were fed ad libitum. Half of the animals in each group were killed after 6 weeks of feeding, and the remaining animals were killed after 12 weeks of feeding. The cholesterol content of the serum and liver was determined for each rat.

A progressive increase in serum and liver cholesterol content was observed in animals fed the stock diet supplemented with cholesterol for 6 months. In animals fed stock diet supplemented with cholesterol and katha for 6 months, the elevation of serum and liver cholesterol levels was significantly lower ($p = .001$) when compared to rats fed stock diet supplemented with cholesterol.

However, at the end of 12 weeks, the increase in serum and liver cholesterol concentrations in rats fed stock diet supplemented with cholesterol and katha was elevated by approximately 50% when compared to rats fed stock diet supplemented with cholesterol only. It was also determined that there was substantially less deposition of lipids in the liver of katha-fed rats. It was concluded that katha had hypocholesterolemic activity in this study, and that it helped prevent fatty degeneration of the liver (Chaudhari and Hatwalne 1973).

Hypoglycemic Activity

Acacia (Not Gum Arabic)

Wassel et al. (1992) studied the hypoglycemic activity of ethanolic extracts of the pod, leaf, stem, old stem, and flower of *Acacia farnesiana L. Willd* using groups of 11 alloxanized diabetic albino rats (weights = 150 to 200 g). To prevent the development of fatal hypoglycemia during the first 12 h after alloxan administration, a 25% glucose solution (5 to 10 ml) was subcutaneously injected at 2 to 3 h intervals. Extract from each plant part (dose = 30 or 50 mg/kg in polysorbate 80) was administered orally to a group of 11 rats, and blood samples were taken at 2 h post administration. Blood samples were collected

prior to treatment in order to estimate the normal blood glucose level of fasting rats.

The hypoglycemic activity of ethanolic extracts of *Acacia farnesiana* stem and pod was considerable following the administration of a 50 mg/kg dose. *Acacia farnesiana* stem and pod caused 21% and 36% reductions in the normal fasting blood sugar level, respectively (Wassel et al. 1992).

Effects on Smooth Muscle

Acacia (Not Gum Arabic)

Wassel et al. (1992) also studied the effect of ethanolic extracts of the pod, leaf, stem, old stem, and flower of *Acacia farnesiana L. Willd* on uterine motility. Rat uteri at various stages of the estrous cycle were suspended in 50-ml baths containing oxygenated Krebs solution; uteri were equilibrated in the solution for at least 90 min. Drugs were added to the water bath and were retained until the highest contraction was achieved.

Normal rhythmic contractions of the isolated uteri were first recorded using a T_2 isotonic transducer and two channel MD_2 oscillograph. Subsequently, the plant extracts (in polysorbate 80) were added to organ water baths at a dose of 50 or 75 mg/50 ml bath. The drug used to induce uterine contraction was then removed by washing the preparation with fresh Krebs solution.

Most of the *Acacia farnesiana* ethanolic extracts stimulated uterine muscular contraction during the estrous cycle and pregnancy. However, some of the extracts had a stimulatory effect on uterine contraction, followed by inhibition (i.e., leaf extract on non-estrus uteri and pod extract on pregnant uterus). The stem extract of *Acacia farnesiana* inhibited contraction of the pregnant uterus (Wassel et al. 1992).

Trivedi et al. (1986) evaluated the bronchodilator activity of *Acacia farnesiana* using the perfused, isolated guinea pig lung. The control guinea pig lung preparation was treated with saline. The unripe pods of *Acacia farnesiana* were collected and dried at room temperature. The glycosidal fraction of the ethyl alcohol extract of coarsely powdered Acacia pods was then isolated, and an aqueous solution of this fraction was tested.

Doses of 2, 5, and 10 μg of the aqueous solution increased outflow in the isolated lung perfusion preparation, indicating that the glycosidal fraction induced a smooth muscle relaxant effect. The same doses also increased outflow following histamine (10 μg)-induced contraction, and the bronchodilator effect was not blocked by propranolol (400 μg). These results suggested that the glycosidal fraction exerted a direct relaxant action on the bronchial muscles. The investigators noted that this effect is not mediated through β-adrenergic receptors.

The vasodilator activity of *Acacia farnesiana* was evaluated in vitro. The glycosidal fraction of the ethyl alcohol extract of coarsely powdered Acacia pods was isolated, and an aqueous solution of this fraction was tested. The hind limb of dogs was perfused through the femoral artery with oxygenated, defibrinated blood in Ringer's solution. Femoral venous outflow was recorded periodically. The control preparation was treated with normal saline.

The aqueous glycosidal fraction induced vasodilation at doses of 2, 5, and 10 μg (% increases in blood flow/min of 21.4, 20.86, and 24.3, respectively; $n = 5$). Vasodilation was not blocked following the addition of any of the following agents: chlorphenaramine maleate (20 μg), atropine (20 μg), or propranolol (400 μg). Study results indicated that the glycosidal fraction of *Acacia farnesiana* had a smooth muscle relaxant effect. The investigators noted that this effect was not mediated through cholinergic or H_1 receptors (Trivedi et al. 1986).

Anti-Inflammatory Activity

Acacia (Not Gum Arabic)

The anti-inflammatory activity of *Acacia farnesiana* was evaluated in vitro. The glycosidal fraction of the ethyl alcohol extract of coarsely powdered Acacia pods was isolated, and an aqueous solution of this fraction was tested. The effect of this fraction on chemically induced edema of the rat hind paw was evaluated according to the method of Winter et al. (1962). The glycosidal fraction inhibited carrageenin and formaldehyde induced inflammation of the rat hind paw in vivo (% inhibition of 38.2 and 26.26, respectively; $p < .001$, $n = 10$). It was concluded that this fraction has a promising anti-inflammatory effect (Trivedi et al. 1986).

Oxidative Phosphorylation

Gum Arabic

Bachmann et al. (1978) administered gum arabic twice daily to groups of four rats (weights = 100 to 110 g) at concentrations of 1%, 2%, and 10%, respectively, 5 days per week for 4 weeks. The test substance was suspended in distilled water and administered orally at a dose volume of 0.2 ml/100 g body weight; control rats were given equal volumes of distilled water. The actual doses of gum arabic administered were 2×20, 2×40, and 2×200 mg/kg/day. Groups of four rats were killed by cervical dislocation 16 h after administration of the last dose. Following maceration and homogenization, heart and liver mitochondria were isolated by differential centrifugation. Electron transfer reactions (oxygen consumption) and oxidative phosphorylation were measured polarographically. The hydroxylation of biphenyl was chosen as the assay system for measuring mixed function oxidases of hepatic cell endoplasmic reticulum.

Dose-dependent uncoupling of oxidative phosphorylation was the primary effect on cardiac and hepatic cell mitochondrial function. The damage to cardiac mitochondria progressed as dosing continued. However, hepatic cell mitochondrial function seemed to have gradually returned to normal during the fourth week of dosing.

At the highest administered dose (2×200 mg/kg/day) marked uncoupling of oxidative phosphorylation was observed in the heart and liver after 2 days of dosing. Partial recovery was reported for cardiac mitochondria after the first week of dosing; however, the same degree of uncoupling was noted up to the end of the experiment. Hepatic cell mitochondria were said to have recovered slowly as the experiment progressed. Gum arabic also

caused a progressive inhibition of the biphenylhydroxylase system in the hepatic microsomal fraction (Bachmann et al. 1978).

Lutz et al. (1978) considered these results and investigated whether comparable biochemical effects of gum arabic (USP grade) could also be demonstrated in vivo. The measurement of maximal aminopyrine demethylation as expired CO_2 was deemed a suitable approach for this investigation, which was conducted using female rats of the ZUR SIV-Z strain (weights = 152 to 180 g). Oral dosing with 10% (w/v) gum arabic had no effect on the in vivo demethylation of 4-dimethyl[^{14}C]-aminoantipyrine (Lutz et al. 1978).

Antimicrobial Activity

Acacia (Not Gum Arabic)

The antimicrobial activity of ethanolic extracts of plant organs from *Acacia farnesiana* was evaluated. Extracts were made from the following plant parts: the pod, leaf, stem, old stem, and flower. Bacteria and yeast were cultured and filter paper disks were impregnated with 10 μl of each extract. Each disk (one extract per disk) was then dried and placed on the surface of the inoculated agar medium, and cultures were incubated for 48 h and observed for zones of inhibition. All plant extracts were inhibitory to *Bacillus subtilis* and *Staphylococcus aureus.* Additionally, most of the extracts were inhibitory to *Sarcina lutea*, *Pseudomonas aeruginosa*, and *Escherichia coli*. The plant extracts had no effect on *Mycobacterium phlei* or *Candida albicans* (Wassel et al. 1992).

ANIMAL TOXICOLOGY

Acute Oral Toxicity

Gum Arabic

In an acute oral toxicity study using rabbits (weights and strain not stated), an Acacia Gum LD_{50} of 80 g/kg was reported (Dangerous Properties of Industrial Materials Report 1981).

Acacia (Not Gum Arabic)

Letizia et al. (2000) conducted a study in which the acute oral toxicity of Acacia Farnesiana Extract (from flowers) was evaluated using ten rats (strain not stated). The test substance was administered at a dose of 5.0 g/kg, and animals were observed for 14 days. Necropsy was performed at the end of the observation period.

An LD_{50} of greater than 5.0 g/kg was reported. Signs observed in animals during the study included chromorhinorrhea in five or more animals and isolated instances of the following: tachypnea, chromodacryorrhea, ptosis, lethargy, piloerection, emaciation, ataxia, and respiratory noise. Necropsy findings for the only animal that died included abnormalities of the lungs, kidneys, liver, spleen, and gastrointestinal tract (Letizia et al. 2000).

The Societe Bertin (1987) reported an acute oral toxicity study in which Cire Essentielle Cassie (trade name for Acacia Farnesiana Flower Wax) was evaluated using groups of five rats (males and females) of the OFA Sprague-Dawley IOPS strain. Mean weights for male and female test animals were 219.60 g and 183.60 g, respectively. Control mean weights were 224.0 g (males) and 183.80 g (females). The animals were all approximately the same age (ages not stated). A single 10 ml/kg dose of the product was administered orally to each animal, and followed by a 14-day observation period. Control animals were dosed with corn oil (10 ml/kg). The animals were killed at the end of the observation period and necropsy performed.

Significant changes in general condition (weight changes included) or behavior between test and control animals were not observed. None of the animals died and no test substance-related organ lesions were observed. The test material was classified as innocuous at the dose administered (Societe Bertin 1987).

Biogir S.A. Conseil Recherche (1990a) also reported the acute oral toxicity of Cire Essentielle de fleurs de Mimosa (trade name for Acacia Dealbata Leaf Wax) in a suspension with paraffin oil using five male (178.3 $\pm$ 9.8 g) and five female (172.8 $\pm$ 5.9 g) rats of the OFA Sprague-Dawley strain (SPF). The animals were 2 months old. A single oral dose of 2 g/kg (10 ml/kg) of the product was administered to each animal by gavage, and dosing was followed by a 14-day observation period. Feeding resumed at 4 h post dosing. At the end of the observation period, the animals were killed and gross necropsy performed.

Weight gain was described as normal and no deaths were reported. Additionally, none of the animals had overt signs of central nervous system or neurovegetative system toxicity, and no lesions of organs examined were noted at necropsy. The minimal lethal dose was greater than 2 g/kg (Biogir S.A. Conseil Recherche 1990a).

Acute Dermal Toxicity

Acacia (Not Gum Arabic)

The acute dermal toxicity of Acacia Farnesiana Extract (from flowers) was evaluated using 10 rabbits (strain and weights not stated). A single dose of 5.0 g/kg was administered dermally to each animal, and observations were made over a period of 14 days. Gross necropsy was performed at the end of the observation period. Signs observed during the study were as follows: isolated instances of lethargy, diarrhea, ptosis, and nasal discharge (yellow). Gross observations at necropsy were normal for each animal. An LD_{50} of greater than 5.0 g/kg was reported (Letizia et al. 2000). Skin irritation reactions observed in this study are included in the section on Skin Irritation later in the report text.

Acute Intraperitoneal Toxicity

Gum Arabic

In a study using dogs (number and weights not stated), the intraperitoneal injection of 4.8 g/kg gum arabic did not induce toxicity. However, the same dose killed dehydrated dogs (highest no-effect level = 1.9 g/kg) (FASEB 1973).

Short-Term Oral Toxicity

Gum Arabic

Informatics Inc. (1972) reported a study in which diets containing Gum Arabic were fed to 133 guinea pigs. Except for one diet containing 20% gum arabic, all of the diets contained 15% gum arabic. The animals were fed for periods ranging from three to nine weeks. No toxic effects resulted from the administration of gum arabic.

Groups of rats (number and weights not stated) were fed 15% gum arabic in the diet for 62 days. A cathartic effect was noted. Weight gain, feed efficiency, hematological findings, and organ weights were normal (World Health Organization 1974).

Anderson et al. (1984) fed three groups of three male Albino Wistar rats (weights = 140 to 160 g) diets containing 1%, 4%, and 8% (w/w) gum arabic (Acacia Senegal Gum), respectively, daily for 28 days. A fourth group served as the negative control. At necropsy, hepatic and cardiac tissues were obtained for electron microscopy and microsomal P-450 assays.

No discernible ultrastructural differences were observed between the livers of test (all dietary groups) and control rats; particularly, the mitochondria were normal. Also, no discernible ultrastructural differences were found between the hearts of test (all dietary groups) and control rats. Particularly, both the appearance and concentration of the mitochondria and myofibrils were identical in this comparison. The results of assays of hepatic microsomal protein and cytochrome P-450 for each dietary group indicated that gum arabic did not cause inductive effects. The investigators noted that when induction by active agents (e.g., phenobarbitone) takes place, cytochrome P-450 values are increased by several-fold within a few days (Anderson et al. 1984).

Anderson et al. (1986) fed 10% (w/w) gum arabic (Acacia Senegal Gum) daily for 45 days to Wistar albino rats (99 to 120 g). The number of rats in the study was not stated. The rats were then killed by cervical dislocation while under ether anesthesia. Portions of the jejunum, ileum, and cecum were excised and the ultrastructure of each was evaluated using transmission electron microscopy.

No abnormalities in organelles were observed within cells of the jejunum, ileum, or cecum of rats fed gum arabic. Additionally, neither inclusions nor other pathological changes were detected. It was concluded that no significant ultrastructural differences occurred between experimental and control rats (Anderson et al. 1986).

Cook et al. (1992) evaluated the oral toxicity of gum arabic (Acacia species not stated) using 3-week-old Sprague-Dawley rats (16 males, 16 females). Three days before dosing, mean body weights were 122 g and 125 g for males and females, respectively. The animals were fed gum arabic (dose not stated) daily for 28 days and then killed by exsanguination. Blood samples were obtained for hematological examination and serum analysis the day before animals were killed. Microscopic examination of most organs was performed, which included examination of any tissues that appeared abnormal.

No treatment-related behavioral effects were noted. All values for serum chemistry parameters were within the normal limits for laboratory rats. Mean red blood cell volume values were said to have been within the normal range for Sprague-Dawley rats. No toxicologically significant lesions were noted at microscopic examination (Cook et al. 1992).

Short-Term Intravenous Toxicity

Gum Arabic

Acacia (Gum Arabic) was administered intravenously to three dogs (weights not stated) over a period of 76 days. The number of intravenous injections ranged from 32 to 35 over this period, and the range for the total cumulative dose was 15.7 to 47.7 g/kg. An enlarged liver was observed in the dog that received the greatest cumulative dose; death occurred four months after the last injection. The cause of death was not stated. The remaining two dogs remained in good condition. The results of biopsies performed on the two animals indicated that Acacia was present in the liver 26 months after the last injection (World Health Organization 1974).

In another study, gum arabic was administered intravenously to dogs (number and weights not stated) over a period ranging from 1 to 84 days. Doses ranged from 1 to 2 g/kg. Enlarged livers and swollen kidneys were the most characteristic changes. Similar doses were fatal when administered to two rabbits (weights not stated) (FASEB 1973).

Subchronic Oral Toxicity

Gum Arabic

Anderson et al. (1982) evaluated the subchronic oral toxicity of gum arabic (Acacia Senegal Gum) in two experiments using albino Wistar rats (24 to 28 days old). Body weights prior to initiation of the study were not included.

In the first experiment, groups of 15 male rats were fed gum arabic at concentrations of 0.91% (dietary level = 0.53 g/kg/day), 2.0% (1.08 g/kg/day), 4.3% (2.55 g/kg/day), and 8.6% (5.22 g/kg/day), respectively, for 13 weeks. Groups of 15 female rats were fed concentrations of 0.75% (0.5 g/kg/day), 1.7% (1.05 g/ kg/day), 3.7% (2.6 g/kg/day), and 7.5% (5.31/g/kg/day), respectively. Fifteen males and 15 females served as controls.

In the second experiment, 15 male rats were fed gum arabic at an average concentration of 18.6% (14 g/kg/day) for 13 weeks. Fifteen female rats were fed an average concentration of 18.1% (13.8 g/kg/day). The two control groups consisted of 15 males and 15 females, respectively. Urine and blood samples were obtained during the study. The animals were killed under anesthesia by cervical dislocation at the end of the treatment period and prepared for necropsy.

The results for the two experiments included the reported deaths of two control female rats. Growth rates were not reduced for male or female rats at dietary doses up to 5 g/kg/day (~8.5% gum arabic in diet). At a concentration of approximately 18% in the diet (14 g/kg/day), male rats had a reduced growth rate and

smaller final body weight ($p < .01$). The average weight gain for male rats was 78% of that of controls.

Following the ingestion of gum arabic, 5 g/kg/day, by male rats, kidney weights (absolute and relative to body weight) were reduced ($p < .05$). At the highest dietary doses tested (~18%, 14 g/kg/day), kidney weights for male and female rats were significantly reduced ($p < .01$). Liver weight was reduced in a dose-dependent manner in male rats; the difference between experimental and control groups was not significant at doses of gum arabic less than 5 g/kg/day. No significant differences were observed in urine volume or composition between control and test groups at any of the dietary concentrations of gum arabic tested.

Similarly, no significant hematological changes were observed between test and control groups. At microscopic examination, no alterations were found that were attributable to the ingestion of gum arabic. The only treatment-related alteration noted at necropsy was cecal enlargement in rats of the highest-dose groups (Anderson et al. 1982).

In another study, Anderson et al. (1984) fed four groups of five male albino Wistar rats (weights = 40 to 60 g) diets containing 0.5%, 1.5%, 2.5%, and 3.5% (*w*/*w*) gum arabic (Acacia Senegal Gum), respectively, daily for 91 days. A fifth group served as the negative control. At the end of the feeding period, the animals were killed by cervical dislocation for necropsy. Samples of liver and heart from each treatment group were obtained for transmission electron microscopy. Livers from the remaining rats (two per group) were used for assays of microsomal protein and cytochrome P-450.

Electron microscopic findings for cardiac muscle included no abnormality of myofilaments, no depletion of glycogen reserves, no abnormality of the intracytoplasmic mitochondria or endoplasmic reticulum, no excessive infiltration with lipid, and no evidence of interstitial infiltration. Additionally, no abnormalities were observed with respect to the size, chromatin content, or nucleoli of nuclei. Electron microscopic findings for the liver included no abnormalities in hepatocytes, Kupffer cells, or lining cells of the biliary passages. The mitochondria and nuclei were normal both in appearance and internal structure, and no abnormalities were observed in intracytoplasmic glycogen stores (Anderson et al. 1984).

Skin Irritation

Acacia (Not Gum Arabic)

In an acute dermal toxicity study, Acacia Farnesiana Extract (from flowers) was administered dermally (single dose of 5.0 g/kg) to 10 rabbits, after which animals were observed for 14 days. On day 1, moderate erythema and moderate edema were observed in all ten rabbits (Letizia et al. 2000).

Biogir S.A. Conseil Recherche (1990b) evaluated the skin irritation potential of Cire Essentielle de fleurs de Mimosa (trade name for Acacia Dealbata Leaf Wax) using six New Zealand albino rabbits. The undiluted product (volume = 0.5 ml on an occlusive patch) was applied to scarified skin (clipped free of hair) of the right flank of each animal. The left flank (nonscarified skin) of each animal served as the control. Each patch was secured with a hypoallergenic, microporous adhesive strip and an elastic band (fixed with adhesive tape) that was wrapped around the trunk. Patches were removed after 24 h of contact.

At 24 h and 72 h post application, reactions were scored according to the following grading scales: 0 (no erythema) to 4 (severe erythema [crimson red] with or without eschar [deep injuries] and lesions showing a serious cutaneous reaction such as a burn, a necrosis) and 0 (no edema) to 4 (severe edema [more than 1 mm thick and extending beyond the area of exposure] showing a serious cutaneous reaction such as a burn). Scores for erythema and edema (intact and scarified skin) were determined at 24 and 72 h post application. The scores (intact and scarified skin) obtained were added together and divided by 24 to calculate the primary cutaneous irritation index.

Cire Essentielle de fleurs de Mimosa (Acacia Dealbata Leaf Wax) was classified as a nonirritant (primary irritation index = 0.5) in New Zealand albino rabbits (Biogir S.A. Conseil Recherche 1990b).

Bertin Laboratories (1987) reported on a study in which the skin irritation potential of Cire Essentielle Cassie (Acacia Farnesiana Flower Wax) was evaluated using six New Zealand rabbits. The test substance (in pure form, 0.5 ml under occlusive pad) was applied to intact and scarified skin of the right and left flank, respectively, that had been clipped free of hair. A stretch bandage was then wound around the torso of each animal and secured with adhesive tape. Patches were removed at 24 h post application. Reactions were scored at 24 and 72 h post application according to the following grading scales: 0 (no erythema) to 4 (serious erythema [purple red] with slight scarring [deep lesions] and 0 (no edema) to 4 (serious edema [over 1 mm thick] with a surface area greater than 1 mm^2).

At 24 h, reactions at intact sites were described as somewhat pronounced erythema (2 rabbits [slight erythema, score = 1]; 1 rabbit [highly visible erythema, score = 2]). Very slight edema (score = 1, intact site) was also noted in the rabbit with highly visible erythema. Identical results were reported for scarified skin sites. At 72 h, reactions were observed in one rabbit; slight erythema and very slight edema were observed at intact and scarified sites. Cire Essentielle Cassie (Acacia Farnesiana Flower Wax) was classified as a slight skin irritant (primary cutaneous irritation index = 0.6) (Bertin Laboratories 1987).

Phototoxicity

Acacia (Not Gum Arabic)

The phototoxicity of a 20% solution of Acacia Farnesiana Extract (from flowers) in methanol was evaluated using six SKH:hairless mice. The test substance was applied to a 5-cm^2 area on the back of each animal. At 30 min post application, test sites were irradiated with UV light for 1 h. The light source consisted of a bank of six fluorescent, black light lamps positioned

at a distance of 35 cm, or an Atlas xenon lamp (model Rm 60 or 65 [wavelength: 280 to 320 nm] with a Schott WG320 filter) positioned at a distance of 1 m. Reactions were scored at 4, 24, 48, 72, and 96 h post exposure. No phototoxic effects were observed (Letizia et al. 2000).

Immunological Responses

Studies on immunological responses to gum arabic and Acacia solution/extract are summarized in Table 9.

Acacia (Not Gum Arabic)

Maytum and Magath (1932) reported a series of three experiments that evaluated the allergenicity of an Acacia solution (exact composition not stated). In the first experiment, six rabbits (12 weeks old) were injected intravenously with 50 cc Acacia, and this dose was repeated 5, 12, and 17 days later. At 4 weeks after the last injection, each rabbit was injected intravenously with 2 cc of Acacia.

The rabbits appeared normal during a 1-h observation period following this injection. On the same day, one of the rabbits was injected intravenously with 2 cc of a 50% egg white solution to determine whether exposure to a foreign protein would result in greater sensitivity to Acacia. Acacia (2 cc) was injected intravenously 3 weeks later, and then 3 weeks after this injection at a dose of 15 cc. No signs of anaphylaxis were observed in this animal (Maytum and Magath 1932).

In the second experiment, eight guinea pigs (weights = 300 g) were injected intraperitoneally with a dose of 10 cc, and this dose was repeated 5, 12, and 17 days later. At four weeks after the last dose, two of the animals were injected intravenously with 0.5 cc Acacia.

Typical anaphylactic signs (sneezing and coughing, scratching the nose, and dyspnea) were noted in both guinea pigs after approximately 30 s. The two animals died approximately 3 min after signs were first noted. Two other guinea pigs were injected intracardially with Acacia solution (0.5 cc; exact composition not stated), after which both had milder signs of anaphylaxis. One animal recovered, and the other died after 1 h. The remaining four guinea pigs each received an intraperitoneal injection of Acacia solution (0.5 cc). Mild reactions were noted in two of the animals, and no signs were reported for the remaining two.

A follow-up third experiment was performed to determine whether the guinea pig deaths reported were due to the intravenous method of test substance administration in the second experiment. Four guinea pigs were injected intravenously with 0.5 cc Acacia solution (exact composition not stated), and no deleterious effects were noted. Acacia solution (10 cc) was administered intraperitoneally to eight guinea pigs; four of the animals died within five days after injection.

Seven days later, intraperitoneal injections of Acacia solution (10 cc) were given to the four remaining guinea pigs (from second experiment) that were injected intraperitoneally, the four guinea pigs that were injected intravenously in the first experiment, and four new guinea pigs. Of the four new guinea pigs, two died from peritonitis within 4 days.

Seven days after intraperitoneal injection, the remaining 10 animals from the third experiment were injected intraperitoneally with 10 cc Acacia. Four of the 10 died of peritonitis on the next day. It was stated that Acacia was capable of inducing peritonitis (followed by death) only after intraperitoneal administration.

In total, these authors reported on the results of studies involving a total of 19 guinea pigs (8 guinea pigs from preceding experiment included) that include sensitization induced by Acacia solution (administered parenterally; exact composition not stated) and no anaphylactic signs developed in seven of the animals.

Mild and moderate anaphylactic signs developed in four and three guinea pigs, respectively, and severe signs were noted in two guinea pigs. Three of the 19 guinea pigs died. In addressing the results from the preceding experiments, the investigators noted that anaphylactic sensitivity to Acacia can develop under certain unusual conditions. It was also stated that no danger was associated with an initial dose of Acacia if the solution was properly prepared; however, subsequent doses administered after at least 3 weeks should be given cautiously because of the possibility of anaphylactic reactions (Maytum and Magath 1932).

Aronson and McMaster (1972) sensitized 12 guinea pigs (strain not stated; weights $\approx$ 300 g) via single intra-abdominal injections of 600 mg Acacia (6% solution, 10 ml; composition of solution and Acacia species not stated). The animals were challenged 1 month later with an intravenous injection of 60 mg of the sensitizing sample or other samples of Acacia.

The nonnecrotizing toxicity of Acacia extract was evaluated using germ-free and conventional guinea pigs of the Hartley strain. The ages of the germ-free animals tested were as follows: group A (12 animals, 8 days old), group B (9 animals, 3 weeks old), and group C (6 animals, 12 weeks old). The test substance (40 mg/ml) was suspended in phosphate buffer (pH 7.4, 0.1 M) and applied topically to the cornea of the right eye; phosphate buffer was applied to the cornea of the left eye. For both substances, one drop was applied every half hour for a total of seven applications.

The following three groups of conventional guinea pigs were also treated according to the same procedure: group 1 (six animals, 8 days old), group 2 (seven animals, 12 weeks old), and group 3 (two animals, 7 months old). All animals were killed 30 min after application of the last drop. Additionally, phosphate buffer was instilled into both eyes of two animals (killed when 8 days old), and the same was true for two other animals (killed when 3 weeks old). The eyes were enucleated immediately after all animals were killed. The animals were bled prior to killing, and serum samples were subsequently obtained for determination of antibody or y-globulin. At microscopic examination, a severe inflammatory response was observed in both germ-free and conventional 8-day-old guinea pigs.

TABLE 9
Immunological responses

Test substance	Animals tested	Test procedure	Results	Reference
Acacia solution	6 rabbits (12 weeks old)	Four i.v. injections (50 cc) on days 0, 5, 12, and 17, followed by single i.v. injection (2 cc) 4 weeks after fourth injection	No signs of anaphylaxis	Maytum and Magath 1932
Acacia solution	8 guinea pigs (weights = 300 g)	Four i.p. injections (10 cc) on days 0, 5, 12, and 17 followed by single i.v. injection (0.5 cc) 4 weeks after fourth injection	Anaphylactic signs (sneezing, coughing, dyspnea) in 8 animals; 2 deaths. Milder signs noted in 2 surviving animals injected intracardially (0.5 cc); 1 died. Mild signs also in 2 of remaining 4 survivors injected intraperitoneally (0.5 cc). In a follow-up experiment involving guinea pigs, it was concluded that Acacia was capable of inducing peritonitis (followed by death) regardless of the route of administration, i.p. or i.v.	Maytum and Magath 1932
Acacia solution	19 guinea pigs (8 guinea pigs in preceding study included)	Parenteral administration	No anaphylactic signs (10 animals); mild and fairly severe anaphylactic signs in 4 and 3 animals, respectively; extremely severe signs in 2 animals; 3 of 19 died	Maytum and Magath 1932
Anti-Gum Acacia rabbit serum	5 guinea pigs (weights = 300 to 450 g)	Passive sensitization with 2 ml of serum (i.p. injection), followed by i.v. dose of a homologous gum (1 mg)	3 animals died at 2 to 3 min post injection. The remaining 2 recovered from anaphylactic shock slowly	Partridge and Morgan 1942
7% Gum Acacia solution	Two groups of 10 guinea pigs (weights = 600 to 1000 g)	Injected subcutaneously (5 ml) repeatedly over 7-week period. After 2 weeks of dosing, animals injected with 1 ml *Brucella abortus* vaccine	No deleterious effects on antibody production resulted, as judged by the development of agglutinative and complement-fixing activity in the serum to *Brucella abortus*	Rice 1954a
7% Gum Acacia solution	4 rabbits (weight range = 1800 to 2650 g)	Injected subcutaneously (10 ml) repeatedly over 4-week period. Injected with *Brucella abortus* vaccine 4 days (2 ml) and 8 days (3 ml) later	No deleterious effects on antibody production resulted, as judged by the development of agglutinative and complement-fixing activity in the serum to *Brucella abortus*	Rice 1954a

(Continued on next page)

TABLE 9
Immunological responses *(Continued)*

Test substance	Animals tested	Test procedure	Results	Reference
7% Gum Acacia solution	Two groups of 10 guinea pigs	Group1: Injected subcutaneously (5 ml) repeatedly over 16-day period. Actively sensitized after seven doses and challenged in 3 weeks Group 2: Received 11 subcutaneous injections. Passively sensitized and challenged 48 h later	Group 1: One animal with signs of asphyxia; 8 animals with shock signs; 2 died Group 2: Typical respiratory signs developed; no deaths Both groups: No significant decline in serum-complement activity	Rice, 1954b
7% Gum Acacia solution	Two groups of 10 guinea pigs	Group1: Injected subcutaneously (5 ml) repeatedly over 16-day period. Actively sensitized after seven doses and challenged in 3 weeks Group 2: Received 11 subcutaneous injections. Passively sensitized and challenged 48 h later	Group 1: One animal with signs of asphyxia; 8 animals with shock signs; 2 died Group 2: Typical respiratory signs developed; no deaths Both groups: No significant decline in serum-complement activity	Rice 1954b
6% Acacia solution	12 guinea pigs (weights = 300 g)	Twelve animals sensitized via single intra-abdominal injections of 600 mg Acacia (6% solution, 10 ml). Challenged 1 month later with i.v. injection of solution or other samples of Acacia. Two additional guinea pigs tested subsequently with Acacia from different lot	Twelve animals with anaphylactic shock; 10 died. Two additional guinea pigs sensitized by intra-abdominal injection of 160 mg Acacia with Freund's adjuvant (2 ml of emulsion containing two parts 20% Acacia), followed by i.v. challenge with 60 mg Acacia 1 month later, died of anaphylactic shock	Silvette et al. 1955
Three grades of Gum Arabic (dissolved in 0.15 M NaCl at concentration of 4 mg/ml). One of the grades was derived from food grade gum arabic	Groups of 6 to 8 female CBA mice (6 weeks old)	Mice immunized by injection of the antigen (0.1 mg in 0.05 ml Freund's adjuvant) into footpad. Delayed-type hypersensitivity measured 21 days after primary immunization	Compared to controls, no significant increase in footpad thickness. Antigen-specific hypersensitivity reaction noted for all three grades of gum arabic	Strobel et al. 1982

Gum Arabic (dissolved in 0.15 M saline at concentration of 400 mg/ml)	Two groups of 8 female BDF1 [(C57BL/6] × DBA/2 F_1] mice (6 to 8 weeks old)	Initially dosed with Gum Arabic (80 mg) by intragastric administration. Mice then immunized by injection of 100 μg gum arabic in saline and complete Freund's adjuvant into hindpaw. Delayed hypersensitivity measured at 3 weeks post immunization	Compared to controls, footpad swelling significantly suppressed. Systemic immunological hyporesponsiveness (oral tolerance) developed in mice fed gum arabic	Strobel and Ferguson 1986
Five different samples of Gum Arabic (*Acacia senegal*)	5 groups of 6 to 8 male [(C57BL/6J] × DBA/2 F_1] (BDF_1) mice	Footpad swelling test. Unimmunized male mice injected intradermally with each sample	All but one sample induced footpad swelling at 24 h. Footpad swelling said to have been indicative of nonspecific irritant effect	Strobel et al. 1986
Five different samples of Gum Arabic (*Acacia senegal*), each emulsified in Freund's complete djuvant	5 groups of 30 to 40 [(C57BL/6J] × DBA/2 F_1] mice	Footpad swelling test. Initially, mice immunized with each sample (200 μg per sample) in left hind footpad. Presence of delayed-type hypersensitivity measured	All samples found to be immunogenic. Intradermal challenge after immunization caused significant increase in footpad thickness at 24 h	Strobel et al. 1986
Five different samples of gum arabic (*Acacia senegal*), each emulsified in Freund's complete adjuvant	5 groups of 30 to 40 [(C57BL/6J] × DBA/2 F_1] mice	Test for cross-reactivity. Blood samples obtained from mice in preceding experiment at 3 weeks post immunization. Antibodies assayed using enzyme-linked immunosorbent assay (ELISA)	Except for one sample, assay results indicated that antigens were shared between the samples tested	Strobel et al. 1986
Acacia Extract	Germ-free and conventional guinea pigs of Hartley strain	Acacia Extract (40 mg/ml) applied topically to the right eye	Microscopic examination results: Severe inflammatory response observed in germ-free and conventional guinea pigs (14 animals total, 8 days old). Minimal inflammatory response in germ-free and conventional guinea pigs (13 animals total, 12 weeks old). Inflammatory response most severe in conjunctiva	Aronson and McMaster 1972

The inflammatory response was described as minimal in 12-week-old germ-free and conventional guinea pigs. In the 7-month-old conventional animals, the responses were much more severe than that noted for 12-week-old germ-free animals. This comparison was made because 7-month-old germ-free animals were not available.

The inflammatory response to Acacia was most severe in the conjunctiva and the subconjunctival tissues were relatively free of inflammatory changes. Swelling of superficial epithelial cells of the central cornea and necrosis of a few of these cells were also observed. The severity of inflammatory responses was correlated with serum y-globulin concentrations. The extent of the inflammation induced by Acacia paralleled y-globulin concentrations in germ-free guinea pigs more closely than in conventional guinea pigs (Aronson and McMaster 1972).

Gum Arabic

Five guinea pigs (weights = 300 to 450 g) were passively sensitized with 2 ml of anti-Gum Acacia rabbit serum via intraperitoneal injection. At 24 to 36 h post injection, an intravenous dose of a homologous gum (1 mg) was administered to each animal, and the animals were observed for signs of anaphylaxis. Three guinea pigs died 2 to 3 min after intravenous administration, and the remaining two slowly recovered from shock during the following 2 to 3 h (Partridge and Morgan 1942).

Rice (1954a) evaluated the effect of Gum Acacia (species not stated) on complement and antibody production using two groups of 10 guinea pigs (strain not stated; weights = 600 to 1000 g). The animals were injected subcutaneously with gum arabic (7% solution, 5 ml) on alternate days prior to and during immunization; gum arabic was injected repeatedly over a period of seven weeks. After 2 weeks of dosing, the animals were bled and injected intraperitoneally with 1 ml of *Brucella abortus* vaccine. Three additional injections of this vaccine were made 4 days (2-ml injection), 8 days (3 ml), and 21 days (3 ml) later.

The guinea pigs were bled again one week after the third and fourth doses of vaccine, and all sera were titrated for hemolytic complement and for agglutinative and complement-fixing activity with *Brucella abortus* antigens. Surviving animals were retested for 6 weeks, bled again, injected with a fifth dose of vaccine, and bled for a fourth time 7 days later. Twenty guinea pigs of comparable weight were included in each of the control groups (immunized and non-immunized).

A sharp decline in complement titers was noted in both groups of guinea pigs injected with Gum Acacia. Following seven injections, only 2 of 18 surviving guinea pigs had complement titers over 1000 units per ml (minimum titer = 455). After 14 injections, one of the remaining animals had a titer that approached normal (minimum titer = 385). During the ensuing period, a rise in complement titer to over 1000 units per ml was noted for five guinea pigs and complement titers below 500 units were noted for eight guinea pigs; the reason for these changes in titer was undetermined. In addition to the reductions in complement titer noted in the two groups, antibody and total serum protein production were also reduced. It was determined that no deleterious effects on antibody production resulted, as judged by the development of agglutinative and complement-fixing activity in the sera to the bacterial antigen *Brucella abortus* (Rice 1954a).

Rice (1954a) also evaluated the effect of Gum Acacia (species not stated) on complement and antibody production using four rabbits (weight range = 1800 to 2650 g). This experiment is from the study summarized in the preceding paragraph. The rabbits were injected subcutaneously with a 7% solution of Gum Acacia (10 ml) every other day for 4 weeks. All rabbits were bled on the 15th day and immunized with 1 ml *Brucella abortus* vaccine. The vaccine was also injected 4 and 8 days later in 2-ml and 3-ml volumes, respectively. The rabbits were bled again seven days after the third dose of vaccine. Untreated rabbits (immunized) and nonimmunized rabbits served as controls.

In contrast to the effects noted in guinea pigs in the preceding study, Gum Acacia did not appreciably lower complement activity. The authors concluded that no deleterious effects on antibody production resulted, as judged by the development of agglutination and complement-fixing activity in the sera to the bacterial antigen *Brucella abortus* (Rice 1954a).

In another study, Rice (1954b) evaluated complement titers in guinea pigs (strain and weights not stated) that were either actively or passively sensitized to a 7% solution of Gum Acacia. Ten guinea pigs were injected subcutaneously with 16 doses (5 ml per dose) of a 7% Gum Acacia (species not stated) solution over a period of 16 days. The animals were actively sensitized after 7 doses, and the nine survivors were bled, challenged, and rebled in 3 weeks.

Signs of asphyxia were reported for one of the nine survivors; this animal survived for more than 3 h. The other guinea pigs became excited shortly after challenge, running around wildly and squealing (shock signs); two eventually died. An additional 10 guinea pigs that had received 11 injections of Gum Acacia solution were passively sensitized, bled, and challenged 48 h later. Typical respiratory signs developed; none of the animals died. No significant decline in serum-complement activity was detected in animals challenged shortly after passive sensitization or in actively sensitized Gum Acacia-treated guinea pigs; however, a decline in this activity was noted. Additionally, in both sensitized groups, initial excitement followed by fatigue and weakness were the most striking clinical signs (Rice 1954b).

Silvette et al. (1955) reported that anaphylactic shock resulted in each of the 12 guinea pigs sensitized via intra-abdominal injection of 160 mg Acacia with Freund's complete adjuvant (FCA) (2 ml of emulsion containing two parts 20% Acacia). Ten guinea pigs died. Two additional guinea pigs were sensitized via intra-abdominal injection of 160 mg Acacia with FCA (2 ml of emulsion containing two parts 20% Acacia). This Acacia sample was from another lot. The animals were challenged intravenously with 60 mg Acacia 1 month later. Typical anaphylactic death was reported for both guinea pigs.

The results of this experiment as well as additional experiments (rabbits and guinea pigs) in this study collectively indicated that four different lots of Gum Acacia were equally effective as immunizing, sensitizing, and anaphylactogenic and desensitizing antigens, based on the results of cross-precipitin tests and cross-anaphylaxis experiments (Silvette et al. 1955).

Antibodies directed against gum arabic (species not stated) have been isolated using affinity chromatography on AH-Sepharose 4B containing gum arabic ligands. These antibodies were induced in rabbits immunized with gum arabic in FCA. It was determined that the antibodies were anti-carbohydrate antibodies with specificity for certain carbohydrate units of the gum arabic. The results of chemical modification and inhibition experiments indicated that 4-α-L-arabinofuranosyl-D-glucuronic acid units of the polysaccharide were the major immunodeterminant groups (Pazur et al. 1986).

Blood group antigens have been demonstrated in gum arabic (species not stated). The following substances were identified using an agglutinin inhibition test of mild hydrolyzed gum arabic: B, C (of ABO blood group system) and H substances (of H blood group system) and Le^a ($Lewis^a$ antigen, in Lewis blood group system). The results of a revised latex agglutination technique indicated the presence of P and S (of MN blood group system) as well as the substances mentioned in the preceding statement. Elution processes, using sensitized and agglutinated latex or kaolin particles, resulted in the identification of B, H, and Le^a substances in gum arabic; the elution of anti-P and anti-S did not occur (Matsuzawa 1968).

Narita (1985) reported the isolation of high-titer anti-Gum Arabic sera obtained from rabbits injected with gum arabic (species not stated). The antisera had cross-reactivity with the $Lewis^a$ antigen (Le^a), as measured by both a single diffusion tube test and the Ouchterlony test (Narita 1985).

Strobel et al. (1982) evaluated the allergenicity of three grades of gum arabic using female CBA mice (6 weeks old; six to eight mice per group). The grades of gum arabic tested were as follows: (1) processed gum arabic recovered by spray-drying from a solution of commercial food grade gum arabic after filtration to remove sand, etc., and after heat treatment to effect pasteurization; (2) finely powdered natural gum arabic of poor commercial quality giving solutions of a dark red-brown color; (3) finely powdered natural gum arabic of very high quality, giving essentially colorless solutions.

The gum exudates were dissolved in 0.15 M NaCl at a concentration of 4 mg/ml by incubation at 37°C for 16 h. The resulting solution was sterilized by irradiation. The mice were immunized by injection of the antigen (0.1 mg in 0.05 ml of FCA) into the left hind footpad. At 21 days after primary immunization, delayed-type hypersensitivity was measured using a skin test. In this test, the antigen (0.1 mg dissolved in 0.15 M saline in volume of 0.05 ml) was injected intradermally into the plantar side of the right footpad of anesthetized mice. Using a micro caliper, footpad thickness was measured in triplicate immediately before intradermal injection and 24 h later. For controls, footpad swelling was measured before and after antigen injection into the footpad of nonimmunized mice, and before and after saline injection into the footpad of immunized mice. All mice were killed one week after the skin tests. The animals were bled and serum separated and decomplemented.

The intradermal injection of antigen into nonimmunized, control mice (four mice per antigen) did not induce significant footpad swelling at 24 h. Similarly, the intradermal injection of saline into immunized control mice did not cause a significant increase in footpad thickness. However, compared to the control, significant positive responses were noted in mice of the test groups ($p < .01$), indicating an antigen-specific hypersensitivity reaction for all three gum arabic specimens that were tested. A comparison of results for the three grades of gum arabic indicated that footpad swelling in mice immunized and tested with the dark, red-brown grade was significantly greater ($p < .005$) when compared to the colorless grade (Strobel et al. 1982).

Strobel and Ferguson (1986) studied the immunological activity of gum arabic (species not stated) using two groups of eight female BDF1 [(C57BL/6] $\times$ DBA/2)F_1] mice (6 to 8 weeks old). A finely powdered sample of gum arabic was dissolved in 0.15 M saline at a concentration of 400 mg/ml. Each of eight mice was then dosed with gum arabic (80 mg) by intragastric administration. Control mice were dosed with saline. At 7 days post dosing, the mice were immunized by injecting a saline solution of 100 μg gum arabic emulsified in an equal volume of FCA (total volume injected = 0.05 ml) into the left hind footpad.

Control mice were immunized with 0.15 M saline in FCA. Prior to and 3 weeks after immunization all mice were bled and decomplemented sera were tested for anti-Gum Arabic antibodies by a micro-ELISA (enzyme-linked immunosorbent assay) technique.

Delayed-type hypersensitivity was also measured (skin test) at 3 weeks post immunization. The mice were anesthetized and 0.1 mg gum arabic (in volume of 0.05 ml) was injected intradermally into the right footpad. Footpad thickness was measured in triplicate immediately before intradermal injection and 24 h later. As controls, footpad swelling was measured before and after gum arabic was injected into the footpad of saline/adjuvant-immunized animals, as well as before and after saline was injected into the footpad of mice immunized with gum arabic.

Footpad swelling was negligible in both control groups. Antibodies were not detected in the serum of mice that were bled before systemic immunization. Serum antibodies were identified in five of eight control (saline prefed) mice after systemic immunization. However, antibodies were not detected in the serum of mice that were prefed with gum arabic. Regarding delayed-type hypersensitivity, a similar pattern was noted. Positive skin tests were reported for all saline-prefed mice. However, footpad swelling in mice prefed with gum arabic was significantly suppressed. Test results indicated that systemic immunological hyporesponsiveness (oral tolerance) developed in mice that were fed gum arabic (Strobel and Ferguson 1986).

Strobel (1986) evaluated the immunogenicity, cross-reactivity, and nonspecific irritant properties of gum arabic (Acacia Senegal Gum) using male mice (6 to 8 weeks old) of the [(C57BL/6J × DBA/2F$_1$] (BDF$_1$) strain. Nonspecific irritant properties were assessed in the foot pad swelling test using control groups of nonimmunized mice. Immunogenicity was evaluated in an in vivo footpad swelling test, and cross-reactivity was assessed by secondary antibody response.

The following gum arabic samples (identified as samples A, B, C, D, and E) were tested in each experiment: (1) Sample A (sodium arabate) resulted from the neutralization of sample C with sodium hydroxide. (2) Sample B resulted from three successive precipitations of sample C from aqueous solution with acidified ethanol. (3) Sample C, gum arabic, was a water-soluble polysaccharide containing rhamnose, arabinose, glucuronic acid, and galactose. (4) Sample D was defined as powdered food grade natural gum arabic. (5) Sample E was obtained by exhaustive ethanolic extraction of sample D. In the nonspecific footpad swelling test, five groups (six to eight mice per group) of nonimmunized male mice were injected intradermally with the five samples, respectively.

Sample A did not induce significant swelling at 24 h; however, samples B, C, and D increased, but only slightly, nonspecific swelling ($p < .05$). Sample E induced the greatest extent of footpad swelling. These results (footpad swelling) were indicative of a nonspecific irritant effect.

In a second experiment, five groups (30 to 40 mice per group) of mice were immunized with the five gum arabic samples (200 μg per sample), respectively, in the left hind footpad. Each gum arabic sample was emulsified in FCA prior to immunization. Control mice (30 to 40 mice) were immunized with saline in FCA. At 21 days post immunization, the presence of delayed-type hypersensitivity (specific cell mediated immunity) was measured in the footpad swelling skin test.

All gum arabic samples were immunogenic in this test. In each case, intradermal challenge after immunization caused a significant increase in footpad thickness at 24 h. In the test for cross-reactivity, blood samples were obtained from mice that had been immunized and tested (footpad swelling test) 3 weeks after immunization. Antibodies were assayed by an ELISA.

Assay results indicated that antigens were shared between all of the samples, except for sample E. Mice immunized with sample A had significant reactions when tested with samples A, B, C, and D. The greatest nonspecific swelling was produced by samples B and C (Strobel et al. 1986).

GENOTOXICITY

Gum Arabic

Both in vitro and in vivo studies on the mutagenicity of gum arabic described as gum arabic, Acacia, or Gum Acacia are summarized in Table 10. Although a few positive results are described, most studies were negative for genotoxicity.

UV Damage Repair

Acacia (Not Gum Arabic)

Jain et al. (1987) evaluated the effect of *Acacia arabica* on UV-induced damage in the WP-2 strain of *Escherichia coli*. Cultures were irradiated with UV light (1.5 J/m^2/s) for 15 s, with intermittent stirring. The bark of *Acacia arabica* was extracted with methanol and the extract was added to cultures at a concentration of 5 mg/plate. The revertants and viable cells were counted after incubation for two days at a temperature of 37°C.

Compared to control cultures exposed to UV light (mean number of revertants per plate = 216), the mutagenic activity of UV light was reduced in cultures dosed with *Acacia arabica* extract. The mean number of revertants per plate in test cultures was 34. The survival for control and test cultures was 100% and 70.6%, respectively. The investigators stated that the decrease in UV-induced mutagenicity in the presence of Acacia could have been due to some enzymatic action that reverted the formation of pyrimidine dimers (Jain et al. 1987).

Effect on Genotoxicity of Other Agents

Gum Arabic

The effect of 3% gum arabic (solvent) on the mutagenicity of 4-nitroquinoline-*N*-oxide was evaluated using results from the bone marrow micronucleus assay. Based on an analysis of time-response and dose-response data on 4-nitro-quinoline-*N*-oxide, it was determined that the mutagenicity of this chemical was six times greater in gum arabic when compared to test results for the chemical in DMSO. When the mutagenicity of other chemicals, such as mitomycin C, was evaluated using different solvents, no solvent effect on mutagenicity was observed. The investigators concluded that no clear relationship existed between the solvent used and the mutagenicity observed (Katz et al. 1981).

Carcinogenicity

Gum Arabic

No evidence of carcinogenicity was noted in rats dosed intraperitoneally with gum arabic (1.75% or 7% in saline or water) three times per week for up to 15 weeks. Based on the data presented, it was difficult to ascertain the size of the dose administered. The doses administered were on the order of several hundred mg/kg. Also, no evidence of carcinogenicity was found in a similar study using mice (doses injected not stated) (FASEB 1973).

Gum arabic gruel was injected intramediastinally (single dose) into five (0.5 ml dose of test substance) and 10 (1 ml dose) guinea pigs. The animals (strain not specified) ranged in weight from 220 to 450 g and were 4 to 10 months old. Neoplasms were not observed in any of the guinea pigs either at necropsy or at microscopic examination of tissue. On the average, the animals survived from 1200 to 1490 days (Tlolka-Pluszczyk 1970).

Melnick et al. (1983) studied the carcinogenicity of gum arabic using 4-week-old F344 rats (50 males, 50 females) and

TABLE 10
In vitro and in vivo mutagenicity of Gum Arabic, as Gum Arabic, Acacia, and Gum Acacia

Test substance	Strains/cells/animals tested	Test procedure	Test results	References
		Gum Arabic		
Bacterial Cell Test Systems				
Gum Arabic	*Saccharomyces cerevisiae* D4	Host-mediated assay for mitotic recombination (Gabridge and Legator 1969); test concentration of 5% w/v if no lethal effects observed	Not mutagenic	Maxwell and Newell 1974
Gum Arabic	*Salmonella typhimurium* G-46 and TA-1530	Ames test (Ames 1971)	Not mutagenic	Maxwell and Newell 1974
Gum Arabic (in DMSO)	*Salmonella typhimurium* TA1535, TA1537, and TA1538 *Saccharomyces cerevisiae* D4	Plate and suspension assays with and without metabolic activation. Plate test concentrations up to 3.3%. Suspension assay concentrations up to 0.36%	Not mutagenic with or without metabolic activation	Litton Bionetics, Inc. 1975
Gum Arabic	*Saccharomyces cerevisiae* D3	Plate test (Brusick 1973)	Not mutagenic	Green 1977
Gum Arabic (in 0.067 M sodium phosphate buffer)	*Salmonella typhimurium* TA1535, TA1537, TA1538, TA98, and TA 100 *E. coli* WP2 (uvrA)	*Salmonella*/microsome assay with and without metabolic activation; concentrations up to 10,000 μg/plate	Not toxic or mutagenic	SRI International 1980
Gum Arabic (in water)	*Bacillus subtilis* M 45 Rec^- and H 17 Rec^+	Spore rec-assay (with and without metabolic activation) for DNA-damaging activity	Not mutagenic	Ishizaki and Ueno 1987
Gum Arabic (in 0.067 M potassium or phosphate buffer)	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, and TA1538 *Escherichia coli* WP2	*Salmonella* strains tested in plate incorporation assay (Ames et al. 1975) with and without metabolic activation; doses up to 10 mg/plate. *E. coli* tested according to modification of plate incorporation assay at same doses	Not mutagenic with or without metabolic activation	Prival et al. 1991
Gum Arabic	*Salmonella typhimurium* TA100, TA1535, TA1537, TA97, and TA98	Modification of preincubation procedure by Haworth et al. (1983) with and without metabolic activation. Cultures incubated with 0.05 ml gum arabic	Not mutagenic with or without metabolic activation	Zeiger et al. 1992
Mammalian Cell Test Systems				
Gum Arabic	Diploid human embryonic lung (WI-38) cells	Cytogenetics assay; concentrations up to 1000 μg/ml culture if no cytotoxicity observed at this level. Anaphase analyses according to procedure of Nichols et al. (1971)	Response classified as "slight positive." No definite abnormal anaphase figures observed	Maxwell and Newell 1974

(Continued on next page)

TABLE 10
In vitro and in vivo mutagenicity of Gum Arabic, as Gum Arabic, Acacia, and Gum Acacia *(Continued)*

Test substance	Strains/cells/animals tested	Test procedure	Test results	References
Gum Arabic	WI-38 human embryonic lung cells	Test methodology not stated	Chromosomal aberrations induced in anaphase	Green 1977
Animal Test Systems				
Gum Arabic	Male albino rats (weights 200 g)	Acute and short-term in vivo cytogenetics assays. Doses up to maximum tolerated dose administered. Cytogenetic evaluations on bone marrow cells in metaphase	No significant positive response, but may have been a slight positive response. Further tests and detailed statistical evaluation needed to confirm this possibility	Maxwell and Newell 1974
Gum Arabic	Male and female Swiss mice (10 to 12 weeks old; weights = 25 to 30 g)	Dominant lethal test. Male mice dosed orally with 1% gum arabic prior to mating	No dominant lethal effect	Kar et al. 1984
Gum Arabic	NMRI mice (weights between 30 to 35 g)	Micronucleus test (bone marrow smears). Mice dosed i.p. with 3% gum arabic	Not genotoxic	Wild et al. 1985
Gum Arabic	Male NMRI mice (weights between 30 to 35 g)	Intrasanguineous host-mediated assay. *Salmonella typhimurium* strain TA 98 culture (0.1 ml) injected into tail vein. Intravenous injection followed by oral dose of 3% gum arabic	Not mutagenic to strain TA 98	Wild et al. 1985
	C57BL virgin female mice	Mouse coat color spot test (transplacental mutagenicity test). Gum Arabic (3%) injected i.p. after mating. Spots classified as relevant caused by mutations at heterozygous coat-color loci	Not mutagenic	
	C57BL mice	Mouse melanocyte test—Used to detect somatic mutations that affect the morphology of pigment cells. Pregnant females received i.p. injections of 3% gum arabic on 16th day after detection of vaginal plug	Not genotoxic	
Gum Arabic	Male and female Sprague-Dawley rats (males: 6 to 8 weeks old; females: 10 to 12 weeks old)	Dominant lethal test. Male rats fed concentrations up to 4% w/w gum arabic prior to mating. Number of live and dead implants counted 14 days after midweek of mating	Statistically significant dominant lethal effects in male rats	Sheu et al. 1986

	(SEC × C57BL)F1 and (C3H × C57BL)F1 female mice (10 to 12 weeks old)	Dominant lethal test. Male mice fed diets containing up to 20% gum arabic prior to mating	No evidence of dominant lethal effect	
	(101 × C3H)F1 male mice (8 weeks old)	Heritable translocation test. Male mice fed test diet containing 15% *w/w* gum arabic prior to mating	No reduction in average litter size. Number of translocation-carrying male progeny in test group was comparable to that of control group	
		Acacia		
Animal Test Systems				
Acacia (in water)	Male Swiss-Webster mice (6 weeks old; mean weights between 16 to 32 g)	Micronucleus test (bone marrow smears). Mice dosed with 2% Acacia in water	Not genotoxic	MacGregor et al. 1983
Acacia	Inbred female Chinese hamsters (*Cricetulus griseus*) (weight range, 26 to 32 g)	Assay for sister chromatid exchanges. Hamsters dosed i.p. or orally with 10% Acacia (dose volume = 10 ml/kg)	Mean number of sister chromatid exchanges not significantly different, compared to control hamsters dosed with 0.9% normal saline	Neal and Probst 1983
Gum Acacia	Male Swiss mice (6 to 8 weeks old)	Chromosomal aberrations and sperm-head morphology assays. Mice dosed with 5% Gum Acacia by gavage (volume per dose = 0.5 ml)	No statistically significant differences in frequency of chromosomal aberrations and incidence of sperm head abnormalities, compared to control (distilled water) group	Prasad et al. 1987
Gum Acacia	Male Swiss albino mice (8 weeks old)	Micronucleus test (bone marrow smears). Mice dosed orally with 5% Gum Acacia	The ratio of polychromatic erythrocytes to monochromatic erythrocytes (P/N ratio) was slightly higher, compared to mice dosed with water	Pentiah et al. 1989
Acacia	Male ICR mice (7 weeks old; weights between 28 and 32 g)	Micronucleus test (bone marrow smears). Mice dosed with 10% Acacia by gavage (volume per dose = 0.02 ml/g body weight	Not genotoxic	Parton et al. 1988
Acacia	Male ICR mice	Micronucleus test (bone marrow smears). Mice dosed with 10% Acacia by gavage (volume per dose = 20 ml/kg)	Not genotoxic	Parton et al. 1990

4- to 5-week-old $B6C3F_1$ mice (50 males, 50 females) in a 2-year chronic study. Both male and female rats were divided into high- and low-dose groups. Low-dose animals were fed gum arabic at a concentration of 25,000 ppm in the diet and high-dose animals were fed 50,000 ppm. Test diets were fed for 103 consecutive weeks, followed by 1 to 2 weeks of feeding of the basal diet. Control mice (50 males, 50 females) and rats (50 males, 50 females) were fed the basal diet only according to the same schedule. Moribund animals and animals that survived to the end of the study were killed using carbon dioxide and necropsied. Tissues were preserved for histopathologic evaluation.

Changes in mean body weight for male and female rats were comparable to those of the respective control groups throughout the study. Slight decreases in body weight (7% to 13%) were observed in female rats. Compared to controls, consistent differences in mean body weight were noted for female mice of the high dose group (50,000 ppm in diet). No significant differences were found in survival between experimental mice or rats when compared to the respective control groups.

Neoplasms were observed only in male rats, and were diagnosed as malignant lymphomas or leukemia/lymphoma. The incidences of malignant lymphomas for control, low-dose (25,000 ppm gum arabic), and high-dose (50,000 ppm gum arabic) experimental groups of male rats were as follows: 4/50 (low-dose), 1/50 (high-dose), 8/50 (concurrent controls), and 31/1066 (historical controls). Compared to the concurrent control group, a significant decrease ($p < .05$) in tumor incidence was observed in the high dose group, and this was the only statistically significant finding for this neoplasm.

The incidences of neoplasms classified as leukemia/lymphoma in control, low-dose (25,000 ppm gum arabic), and high-dose (50,000 ppm gum arabic) groups of male rats were: 19/50 (low-dose), 16/50 (high-dose), 18/50 (concurrent controls), and 238/1066 (historical controls). Compared to concurrent controls, no statistically significant differences were observed in the incidence of tumors of this type.

No significant changes were observed in the incidence of primary neoplasms in mice that were fed gum arabic in the diet at concentrations of 25,000 or 50,000 ppm. Based on the preceding results, the investigators concluded that gum arabic was not carcinogenic in F344 rats or $B6C3F_1$ mice of either sex (Melnick et al. 1983).

Cocarcinogenicity

Gum Arabic

Vogel and Zaldivar (1971) studied the cocarcinogenicity of Gum Acacia using male rats of the Buffalo strain (6 to 10 weeks old). Thirty-four rats were exposed to fission neutrons (single exposure of 300 to 364 rads; whole-body irradiation), followed by three intraperitoneal injections (0.5 ml per injection) of a 7% solution of Gum Acacia in 0.85% sodium chloride weekly for 23 weeks.

A second test group (30 rats) was irradiated after treatment with Gum Acacia according to the same procedure. Three groups of rats served as controls: one of the control groups (50 rats) was exposed to fission neutrons only. Two additional control groups consisted of 40 rats injected intraperitoneally with 7% Gum Acacia only (according to test group protocol) and an untreated control group of 79 rats.

No significant neoplasm incidence was present in the two control groups. However, the survival time for the 40 control rats injected with Gum Acacia (554.8 $\pm$ 39.4 days, $n = 30$) was significantly shortened when compared to untreated controls (669.2 $\pm$ 19.0 days, $n = 58$). Increases in hepatic, gastric, and intestinal neoplasms were noted in the first test group (34 rats; neutron exposure followed by Gum Acacia injections), when compared to the group of 50 rats exposed to fission neutrons only.

Except for gastric neoplasms, these differences in neoplasm incidence were considered small and probably not significant. It is important to note that no gastric neoplasms were observed in the 50 rats exposed to fission neutrons only, whereas, 20% of the 34 test rats had gastric cancers. No explanation for this difference was given.

Tissues of 28 of the 34 test rats in this group were subjected to complete histopathological analysis after necropsy. Similarly (compared to fission neutrons control group), no gastric neoplasms were noted in the group of 30 rats treated with Gum Acacia and then exposed to fission neutrons. The investigators stated that this finding could have been due to the small number of rats ($n = 14$, compared to $n = 28$ in other test group) subjected to complete histopathological examination after necropsy.

The authors stated that the data presented in this study suggest that Gum Acacia might be considered a "potentiator" for carcinogenesis (Vogel and Zaldivar 1971).

Gum arabic has been reported to increase the number of metastases in mice injected intraperitoneally with Ehrlich ascites carcinoma cells. The carcinoma cells were injected 6 or 24 h after the mice were injected intravenously with gum arabic. However, under some conditions, ascites tumor formation was inhibited (Osswald 1968).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Gum Arabic

Studies on the reproductive and developmental toxicity of gum arabic are summarized in Table 11 and discussed below.

The antifertility activity of Gum Acacia (1 ml in water) was evaluated using 10 female rats (strain and weights not stated). The test substance was administered by stomach tube daily for a period of 5 days after mating. After performing laparotomy on anesthetized dams, the number of fetuses was counted on the 10 day of pregnancy. The average number of implants per rat was 7.8. The percentage of rats with no implant was 0 (Sabir and Razdan 1970).

In a study by the Food and Drug Research Laboratories (1972), the teratogenicity of gum arabic was evaluated using six

TABLE 11
Reproductive and developmental toxicity studies

Test substance	Animals/cells tested	Test procedure	Test results	References
		Acacia		
10% aqueous Acacia solution	9 Little Dutch female rabbits (average weight between 2.1 kg)	After mating, 10% aqueous Acacia solution administered orally on day 0 and the following 6 days	Normal microscopic variations in blastocysts reported: minor trophoblastic vacuolation, trophoblastic degeneration granules, and trophoblastic knob formations	Schardein et al. 1965
Gum Acacia	10 female rats	Gum Acacia (1 ml in water) administered orally during 5-day period after mating	No antifertility activity. Average number of implants per rat = 7.8	Sabir and Razdan 1970
Gum Acacia	Two groups of 5 male albino Wistar rats (4 months old; weights between 180 to 200 g)	First group dosed orally (dose = 1 ml) daily for 24 days. Second group dosed orally (dose = 1 ml) for 48 days	No suppression of spermatogenesis	Akbarsha and Manivannan 1973
4% Gum Acacia	6 Haffkine albino rabbits (weights between 175 to 225 g)	Males dosed orally daily for 28 days and mated with untreated females for total of 12 weeks	No statistically significant difference in number of pregnant females. No antifertility effect in males	Yegnanarayan and Joglekar 1978
	Adult female rabbits (weights between 1 to 2 g)	Dosed orally with 4% Gum Acacia for two days	No inhibitory effect on ovulation	
	Female albino rats (weights between 150 to 200 g)	4% Gum Acacia administered orally to 10 females over period of two estrus cycles, followed by mating with males during proestrus phase of third estrus cycle (short-term experiment). 4% Gum Acacia administered orally to 6 females over period of 6 estrus cycles, followed by mating during proestrus stage of 7th estrus cycle	No significant differences in mating (number of females inseminated) between experimental and control groups. No significant changes in duration of estrus cycles after dosing	

(Continued on next page)

TABLE 11
Reproductive and developmental toxicity studies *(Continued)*

Test substance	Animals/cells tested	Test procedure	Test results	References
	10 female rats (weights between 150 to 200 g)	Females dosed orally with 4% gum arabic on days 1 to 7 of pregnancy	No statistically significant difference in average litter sizes between experimental and control groups, indicating that fetal resorption did not occur	
	10 female rats (weights between 150 to 200 g)	Females dosed orally with 4% gum arabic on days 10 to 16 of pregnancy	No statistically significant differences in number of pups delivered between experimental and control groups	
5% Gum Acacia	9 Syrian golden hamsters (8 weeks old; weights between 80 to 100 g)	Dosed orally with 5% Gum Acacia (dose volume = 0.1 ml/10 g body weight) daily for 54 days	All of the hamsters produced morphologically normal sperm	Waller et al. 1983
1% Gum Acacia	10 female Charles Foster rats (90 days old; weights between 200 ± 20 g)	Administered daily at dose of 50 mg/kg/day during the period of organogenesis	No gross or visceral defects	Sethi et al. 1989
		Gum Arabic		
1% aqueous suspension or mucilage prepared from gum arabic	NMRI mice	1% aqueous suspension or mucilage prepared from gum arabic injected intraperitoneally (single injection or series of 5 injections), subcutaneously (5 injections), and administered orally (5 times) between the 11th and 15th day of gestation	No lethal effects on fetuses	Frohberg et al. 1969
Gum Arabic	Adult female albino CD-1 outbred mice (4 groups). Most groups contained 22 to 23 mice	The four groups of mated mice received oral doses of 16, 75, 350, and 1600 mg/kg on days 6 through 15 of gestation	The number of abnormalities observed in soft or skeletal tissues of fetuses did not differ from the number occurring spontaneously in sham-treated controls	Food and Drug Research Laboratories 1972
	Groups of female rats, rabbits, and hamsters	Oral doses of 16, 75, 350, and 1600 mg/kg on gestation days 6 through 10 (hamsters) and 6 through 15 (rats). Oral doses of 8, 37, 173, and 800 mg/kg in corn oil on days 6 through 18 of gestation (rabbits)	The number of abnormalities observed in soft or skeletal tissues of fetuses did not differ from sham-treated controls	

Gum Arabic (Acacia Senegal Gum)	Groups of 4-week-old Osborne-Mendel (FDA strain) rats	Groups fed dietary concentrations up to 15% beginning at week 13 prior to mating	Gum Arabic not classified as a reproductive or developmental toxicant in rats	Collins et al. 1987
5% aqueous gum arabic solution	36 female Sprague-Dawley Crl:CDBR rats (~9 months; weights between 207 to 314 g)	Solution administered orally once daily (5 ml/kg/day) on days 6 through 17 of gestation	External, visceral, and skeletal malformations observed were unrelated to dosing with Acacia	Morseth and Ihara 1989a
5% aqueous gum arabic solution	30 male Sprague-Dawley Crl:CDBR male rats (6 weeks old; weights between 181.9 to 226.3 g) 30 female rats of same strain (10 weeks old; weights between 210.9 to 309.9 g)	Solution administered orally to females once daily (5 ml/kg/day) for 14 days prior to mating, throughout the mating period, and through day 19 of gestation or day 21 of lactation. Solution also administered to males prior to and during mating and until animals killed	No treatment-related abnormal estrous cycles. No external, skeletal, or soft tissue malformations	Morseth and Ihara 1989b
Gum Arabic	12 Sprague-Dawley rats (adult males)	Control rats fed 30% gum arabic in the diet for 82 days	No effect on spermatogenesis (all males were fertile)	Huynh et al. 2000

groups of mated adult female albino CD-1 outbred mice. Three of the test groups consisted of 22 to 23 mice per group and received doses of 16, 75, and 350 mg/kg, respectively, on days 6 through 15 of gestation. Doses were administered by oral intubation. The fourth test group of 31 mice was dosed with gum arabic (1600 mg/kg) according to the same procedure. Sham-treated mice (28) served as negative controls, and positive-control mice were dosed with aspirin (150 mg/kg). Mean body weights for the test groups ranged from 30 to 39.7 g and were 31.2 g and 31.8 g for negative and positive controls, respectively.

On day 17, all dams were placed under anesthesia and cesarean section was performed. The numbers of implantation sites, resorption sites, and live and dead fetuses were recorded. The urogenital tract of each dam was examined in detail for anatomical normality. Gross examinations for the presence of external congenital abnormalities were performed on all fetuses. Detailed visceral examinations employing 10× magnification were performed on one-third of the fetuses from each litter. The remaining two-thirds were examined for skeletal defects.

The administration of gum arabic to pregnant mice at doses up to 1600 mg/kg had no clearly discernible effect on nidation or maternal or fetal survival. The number of abnormalities observed in either soft or skeletal tissues of fetuses from test groups did not differ from the number occurring spontaneously in sham-treated controls.

As part of this study, groups of rats, rabbits, and hamsters were dosed with gum arabic according to the following modifications of the above test procedure: doses (indicated above) were administered to hamsters (gestation days 6 through 10), rats (gestation days 6 through 15), and rabbits (gestation days 6 through 18). Cesarean sections were performed earlier on hamsters (day 14) and later on rats (day 20). Positive-control rats and hamsters received a higher dose of aspirin (250 mg/kg). Rabbits were dosed with gum arabic in corn oil (8, 37, 173, and 800 mg/kg, respectively); cesarean sections were performed on day 29. Rabbits were injected with human chorionic gonadotropin (day 0) and artificially inseminated. Mean weights for the dams tested were as follows: 200 to 216 g (24 rats per group), 104.6 to 118.4 g (21 to 24 hamsters per group), and 2.01 to 2.43 kg (15 rabbits per group).

The administration of gum arabic, in corn oil, to pregnant rabbits at doses up to 37 mg/kg (highest dose tested = 800 mg/kg) had no clear effect on nidation or maternal or fetal survival. The number and types of abnormalities observed in fetal soft or skeletal tissues from this group did not differ from the number occurring spontaneously in the sham-treated controls. Of the four test groups of rabbits (15 dams per group), the number of survivors per dose group was reported as follows: 13 rabbits (8.0 mg/kg dose group), 15 rabbits (37.0 mg/kg), 12 rabbits (173.0 mg/kg), and 9 rabbits (800.0 mg/kg). In 173 and 800 mg/kg dose groups, maternal death was preceded by severe bloody diarrhea, urinary incontinence, and anorexia. At necropsy, hemorrhage in the mucosa of the small intestines was the only gross pathological finding (Food and Drug Research Laboratories 1972).

Akbarsha and Manivannan (1993) studied the reproductive toxicity of Gum Acacia using two groups of five male albino rats of the Wistar strain (4 months old; weights between 180 and 200 g). The test substance was administered orally (dose = 1 ml) to the first group daily for 24 days. The second group was dosed (dose = 1 ml) daily for 48 days. Rats in both groups were necropsied 24 h after the last dose.

The testis, epididymis (divided into caput and cauda), seminal vesicle, ventral prostate, and coagulating gland were excised, homogenized, and centrifuged. The supernatant was used for determination of total protein and acid phosphatase (ACPase) and alkaline phosphatase (ALPase) activities. Supernatant obtained from the testes was also used for the determination of glycogen and cholesterol, and lactate dehydrogenase (LDH) activity.

The authors stated that increased glycogen and LDH in the testis are both consequences of spermatogenic arrest, and that decreased ACPase and increased ALPase activities in the testis also reflect the suppression of spermatogenesis. They concluded that Gum Acacia did not suppress spermatogenesis in this study (Akbarsha and Manivannan 1993).

Huynh et al. (2000) used gum arabic as the vehicle control in a study evaluating the effect of triptolide (deterpene triepoxide) on spermatogenesis in adult male Sprague-Dawley rats (12 animals, 90 days old). Control males were fed 30% gum arabic in the diet daily for 82 days. Males in the test group were each fed triptolide at a daily dose of 100 μg/kg body weight. Male and female rats (two females per male) were housed together during the feeding period, after which pregnancy rates were determined. The presence of sperm in morning vaginal smears was used to determine whether or not mating was successful. Any male that impregnated at least one of the females was considered fertile. All 12 control males were fertile, whereas all males fed triptolide in the diet were sterile.

Collins et al. (1987) evaluated the teratogenicity of gum arabic (Acacia Senegal Gum) using groups of 4-week-old Osborne-Mendel (FDA strain) rats. Beginning at 13 weeks prior to mating, the rats were fed gum arabic at concentrations of 1%, 2%, 4%, 7.5%, or 15%, respectively. Another group of rats was fed a control diet. Control and test diets were also fed throughout mating and gestation. After mating was confirmed, females were placed in groups of 41 to 47. The dams were killed on day 20 of gestation.

One female rat (1% dietary group) died during the study. External observations of the dams were unremarkable. One female (7.5% dietary group) did have a cystic ovary and one had lung nodules (15% dietary group). Sporadic nonsignificant increases in body weight were observed in all experimental groups.

The percentage of pregnant females was approximately the same in all experimental groups and controls. Mean numbers of corpora lutea and implants per female were also similar to control values, and the average number of viable fetuses was similar in all groups. No effect was seen in any group with

respect to the mean number of viable males and females. Three litters were totally resorbed, one litter from the control, 1%, and 4% dietary groups. Gum arabic in the diet had no effect on the percentage of females with at least one resorption or with at least two resorptions. The numbers of early and late deaths, singly or combined (as average percentage of resorptions), were similar to control values.

The feeding of gum arabic had no effect on mean fetal body weights and crown-rump lengths. The ingestion of gum arabic also had no effect on the distribution of fetuses by sex. A significant decrease in mean female body weight in the 1% dietary group was noted; however, this observation was deemed a random occurrence. The significant increase in the length of females in the 4% and 7.5% dietary groups was not considered biologically significant.

The investigators stated that because of the large group of animals in this study, small variations in crown-rump length can result in significant effects. Similar numbers of runts were noted among male and female fetuses from all dietary groups, with the exception of no runts among male fetuses in the 1% and 15% dietary groups.

Regarding external variations in live fetuses, spina bifida and exencephaly were observed in two fetuses from the control group. No other terata were observed, and the external variations were distributed randomly. Similar numbers of fetuses with hemorrhages were observed in all dietary groups.

The mean numbers of sternebral variations per litter varied from 4.18 (4% gum arabic dietary group) to 5.09 (15% dietary group) in experimental groups, and the mean number of sternebral variations per litter in the control group was 5.21. The variations included reduced ossification and bipartite, missing, and malaligned sternebrae. No dose-related increases were found with respect to any of the observed sternebral deficiencies, and no significant differences were found between experimental and control groups. The significant decrease in the average number of fetuses with one or more sternebral variations per litter that was observed in the 4% and 7.5% dietary groups was considered a random occurrence. Thus, the ingestion of gum arabic did not affect the incidence of litters with fetuses with sternebral variations.

Skeletal ossification deficiencies were observed in bones other than sternebrae; however, no dose-related differences were observed between experimental and control groups with respect to any variation. Furthermore, no dose-related effect was found on the incidence of variations, fetuses with variations, or litters affected in any of the dietary groups.

Also, no dose-related effect was observed on the incidence of any type of soft-tissue variation. Most of the soft tissue variations involved the kidneys. Additionally, the incidence of soft tissue variations in fetuses from experimental and control groups was similar. The mean numbers of soft tissue variations per litter ranged from 0.30 (15% dietary group) to 0.82 (7.5% dietary group), and the mean was 0.76 per litter in the control group (Collins et al. 1987).

Schardein et al. (1965) administered a 10% aqueous *Acacia* solution by gavage to two groups of nine Little Dutch strain mated female rabbits (average weight = 2.1 kg) at doses of 1.26 and 1.5 ml/kg, respectively. Doses were administered on day 0 and the following 6 days (7 doses per female). Nine untreated rabbits served as negative controls. Blastocysts were removed from the uterine horns at 6.5 days of age, prepared as flat mounts, and then evaluated.

The number of fertile rabbits with blastocysts recovered (eight of nine rabbits) in the 1.26 and 1.5 ml/kg dose groups was the same as that noted for the untreated control group. The mean numbers of blastocysts per rabbit were as follows: untreated controls (5.3 ± 1.2), 1.26 ml/kg dose group (7.0 ± 1.7), and 1.5 ml/kg dose group (5.4 ± 2.2). Normal microscopic variations in blastocysts were reported for test and control groups. These variations included minor trophoblastic vacuolation, trophoblastic degeneration granules, and trophoblastic knob formations (Schardein et al. 1965).

Morseth and Ihara (1989a) studied the teratogenicity of a 5% solution of gum arabic (powder) in distilled water using 36 female Crl: CDBR rats (~9 months old) for which mating had been confirmed. Body weights on gestation day 0 ranged from 207 to 314 g. The solution was administered by gavage once daily (5 ml/kg/day) on gestation days 6 through 17. The dams were necropsied on day 20 of gestation. Fetuses were subjected to external (303 fetuses), visceral (102 fetuses), and skeletal (201 fetuses) examinations.

External variations were not observed in any of the fetuses evaluated; however, external malformations, brachygnathia and rudimentary/short tail, were observed in one fetus. Visceral variations included only two fetuses with increased renal pelvic cavitation. At skeletal evaluation, one fetus had brachygnathia, tail short/rudimentary, abnormal fusion of sternebrae, and vertebral anomaly with/without associated rib anomaly. The external, visceral, and skeletal malformations observed were unrelated to dosing with Acacia (Morseth and Ihara 1989a).

Morseth and Ihara (1989b) evaluated the effect of a 5% solution of gum arabic (powder) in distilled water on fertility and general reproductive performance using 30 male (6 weeks old; weights = 181.9 to 226.3 g) and 30 female (10 weeks old; weights = 210.9 to 309.9 g) Sprague-Dawley Crl: CDBR rats. The solution was administered (oral intubation) to male and female rats once daily (5 ml/kg/day) for 63 days prior to mating, throughout the mating period, and until the animals were killed. Male rats were killed after the females had littered. The oral dosing schedule for female rats was daily for 14 days prior to mating, throughout the mating period, and through gestation day 19 or day 21 of lactation. Fifteen female rats were killed on day 20 of gestation, and the remaining females were allowed to raise their neonates to day 22 postpartum.

No abnormal estrous cycles that were considered treatment-related were observed in any of the females. Twenty-nine of the 30 females became pregnant; the male fertility index was 97%. Mean viability and mean weaning indices were 96% and 98%,

respectively. No external, skeletal, or soft tissue malformations were observed (Morseth and Ihara 1989b).

The reproductive toxicity of 5% Gum Acacia was evaluated using nine male Syrian golden hamsters (8 weeks old; weights = 80 to 100 g). The males were mated with female Syrian golden hamsters in order to confirm fertility. Subsequently, the males were dosed (oral gavage) with 5% Gum Acacia (dose volume = 0.1 ml/10 g body weight) daily for 54 days. The animals were killed 3 days after the last dose. As determined by analysis of testis sections, spermatogenesis was reported for all hamsters. All of the hamsters produced morphologically normal sperm, which were also observed in the epididymis (Waller et al. 1983).

Yegnanarayan and Joglekar (1978) studied the antifertility effects of 4% Gum Acacia in a series of five experiments using male and female rats and female rabbits of the Haffkine strain.

In the first study, six male albino rats (weights = 175 to 225 g) were tested. The rats were dosed orally daily for 28 days using a rubber catheter. Beginning on the first day of feeding, males were mated (one male to two females) with females for 12 weeks. Females were replaced each week of feeding. Additional groups of females were mated with control males dosed with saline according to the same procedure. Vaginal smears were examined daily for the presence of spermatozoa. Pregnant females were surgically observed on the tenth day of pregnancy.

The number of inseminated females (73) was the same in experimental and control groups. The total number of pregnant females in experimental and control groups was 24 and 37, respectively, but this difference was not statistically significant.

In the second experiment, the effect of 4% Gum Acacia on the estrus cycle and mating was evaluated using fertile female albino rats (weights = 150 to 200 g). The experiment was divided into two phases. In the first phase (short-term treatment), 4% Gum Acacia was administered orally to 10 female rats over a period of two estrus cycles, beginning on the day of proestrus. The females were mated singly with males during the proestrus phase of the third estrus cycle. In the second phase (long-term treatment), 4% Gum Acacia was administered orally to six female rats over a period of six estrus cycles, beginning in the proestrus phase. Mating was allowed in the proestrus stage of the seventh estrus cycle. In both the first and second experimental phases, control females dosed with saline were mated with males according to the same procedures, respectively. Results for the first and second phases of this experiment indicated no significant differences in mating (number of females inseminated) between experimental and control groups. Additionally, for both phases, no significant changes were observed in the duration of estrus cycles after dosing.

The third experiment, for determining anti-implantation effects, involved 10 fertile rats (weight range from 150 to 200 g) that were mated in proestrus singly with fertile males. Females were dosed orally with 4% gum arabic on days 1 to 7 of pregnancy. The animals were allowed to deliver normally and litter sizes were recorded. Ten control females dosed with saline were mated according to the same procedure. No statistically significant differences were observed in average litter sizes between experimental and control groups, indicating that fetal resorption did not occur in litters of rats dosed with 4% gum arabic.

The fourth experiment was performed to determine any postimplantation effect of 4% gum arabic using ten fertile rats (weights = 150 to 200 g). Female rats were dosed orally with 4% gum arabic on days 10 to 16 and the number of pups delivered was determined. The rats were observed for vaginal bleeding, indicative of abortifacient activity during pregnancy. Control females were dosed with saline according to the same procedure. One of 10 experimental rats did not have a litter. All control females had litters. No statistically significant differences were observed in the number of pups delivered between experimental and control groups.

In the fifth experiment, the antiovulatory potential of 4% Gum Acacia was evaluated using adult female rabbits (number not stated; weights = 1 to 2 kg). The rabbits were dosed orally with 4% gum arabic for 2 days. Copper acetate (4 mg/kg) was then injected into the marginal ear vein in order to induce ovulation. At 48 h post injection, laparotomy was performed; fresh bleeding points on the ovaries were indicative of ovulation. Control rabbits were pretreated with saline according to the same procedure prior to the injection of copper acetate. After the injection of copper acetate, bleeding points on the ovaries were observed in all control and experimental rabbits. Therefore, the authors concluded that 4% Gum Acacia did not have an inhibitory effect on ovulation (Yegnanarayan and Joglekar 1978).

A 1% aqueous suspension or mucilage prepared from gum arabic had no lethal effects on fetuses of NMRI mice injected intraperitoneally (single injection or series of five injections), subcutaneously (five injections), or administered orally (five times) between the 11th and 15th day of gestation (Frohberg et al. 1969).

The embryotoxicity of 1% Gum Acacia was evaluated using ten Charles Foster rats (90 days old; weights = 200 ± 20 g). The test substance was administered daily at a dose of 50 mg/kg/day during the period of organogenesis. The fetuses were delivered by cesarean section on day 20 of gestation, fixed in Bouin's solution, and examined for visceral and skeletal defects. None of the fetuses had gross or visceral defects (Sethi et al. 1989).

CLINICAL ASSESSMENT OF SAFETY

Absorption, Distribution, and Excretion

Gum Arabic

The FASEB (1973) review stated that there was no evidence of the absorption of intact gum arabic found in a study using infants. Twenty-two infants, 1 to 15 months old, were fed gum arabic (15 to 20 g per day) in milk. No urinary excretion of pentose or significant excretion of gum arabic was observed in the stools.

In a nephrotic patient, 20% of the gum arabic injected intravenously over a period of 6 weeks was excreted in the urine.

Other studies involving patients with nephrosis indicated that intravenously injected Gum Acacia, or some product associated with it, accumulated in the liver and remained in the tissues for several months. Serious disturbances in hemoglobin, white blood cells, and serum proteins, all nonlethal effects, were noted (FASEB 1973).

Ross et al. (1984a) evaluated the excretion of gum arabic and its effect on glucose absorption and routine hematological and biochemical measurements in five healthy male volunteers (30 to 55 years old). All subjects were free of signs of gastrointestinal disease. The study was divided into two time periods, a 7-day control period that was followed by a 24-day treatment period. After an overnight fast, glucose (50 g in 200 ml H_20) was fed to each subject on the first day of the control period. During the 24-day treatment period, gum arabic (25 g in 125 ml 7% dextrose) was ingested daily by each subject. Urine was collected on 1 day of the control period and on 1 day during the 3rd week of the treatment period. Complete 5-day fecal collections were made on days 2 to 6 of the control period and on days 16 to 20 of the treatment period. Pooled stool slurry samples from the five subjects were centrifuged. A precipitate typical of gum arabic was not detected in fecal specimens collected before or after the administration of gum arabic.

The marked increases in breath hydrogen production noted after gum arabic ingestion were indicative of bacterial breakdown of gum arabic in the cecum and colon after 3 weeks of administration. Additional study results are summarized in the following paragraph.

No significant differences in the mean concentration of serum lipids (phospholipids and triglycerides) were noted before and after gum arabic ingestion. However, a significant decrease in serum cholesterol (0.39 mmol/L reduction; $p < .05$) was noted. Also, no statistically significant differences were observed between the mean blood glucose concentration (control) and the glucose concentration after the administration of gum arabic.

Similarly, no significant differences were found in the mean insulin concentration (before versus after gum arabic ingestion). Alanine aminotransferase and aspartate aminotransferase activities were significantly reduced ($p < .0025$; $p < .001$) after gum arabic ingestion; however, both mean values were within the normal limits for the population. Of the 13 biochemical measurements that were estimated in the plasma, these reductions in plasma enzyme concentrations represented the only noted significant changes (Ross et al. 1984a).

Short-Term Oral Toxicity

Gum Arabic

Five healthy male subjects (30 to 55 years old) ingested 25 g gum arabic (Acacia Senegal Gum) daily for 21 days. Toxic effects were not observed during the 21-day period; breath hydrogen concentrations increased only after chronic administration. The fact that gum arabic was not recovered from the feces suggest that it is degraded extensively in the human colon (Anderson 1986).

Short-Term Intravenous Toxicity

Acacia (Not Gum Arabic)

Acacia was administered to nine patients with nephrotic edema over periods up to 8 weeks. The test substance was administered intravenously, and total doses ranged from 80 to 325 g. No signs or symptoms of hepatic enlargement or any other complications were observed. Five of the patients excreted 5.5% to 38% of a single dose in the urine during periods ranging from 10 to 30 days, respectively (World Health Organization 1974).

Skin Irritation

Acacia (Not Gum Arabic)

The skin irritation potential of Acacia Farnesiana Extract (from flowers, 4.0% in petrolatum) was evaluated in a 48-h closed-patch test using 30 healthy male and female volunteers. Skin irritation was not observed (Letizia et al. 2000).

Shaligram and Vakil (1990) evaluated the skin irritation potential of Acacia Concinna Fruit Extract (2% in carageenan base [pH of 6 to 7] or 2% in a shampoo [pH of 7 to 8]) in a use test involving 30 normal subjects. The carageenan base and the shampoo, both without the fruit extract, served as controls. The application procedure was described as a routine half head (wet surface) application of Acacia Concinna Fruit Extract (in carrageenan base or in shampoo). The respective controls were applied to the other half of the head (wet surface). Application of test and control materials was followed by rinsing with warm water at 10 to 15 min post exposure. The scalp of each subject was evaluated for signs of irritation (erythema, edema, or any other reaction) at 24 and 48 h post application.

Neither Acacia Concinna Fruit Extract (2% in carrageenan or 2% in shampoo base) nor the controls induced skin irritation (Shaligram and Vakil 1990).

Skin Sensitization

Gum Arabic

Ivy Laboratories (2000) evaluated the skin sensitization potential of a mascara containing 8.0% Acacia Senegal in a maximization test using 28 healthy adult volunteers (males and females, 18 to 49 years old). Twenty-five subjects completed the study because three withdrew for reasons that were unrelated to the test procedure. During the induction phase, approximately 0.1 ml of 0.25% aqueous sodium lauryl sulfate (SLS) was applied (under an occlusive patch) to each subject. Patches were applied to the upper outer arm, volar forearm, or to the back for 24 h. After patch removal, 0.1 ml of the mascara was applied (under an occlusive patch) to the same site on each subject. Patches remained in place for 48 h, except for weekend applications in which the contact period was 72 h. Sites were observed for signs of irritation at the time of patch removal.

If skin irritation was not observed, an occlusive patch containing 0.25% aqueous SLS was applied to the same test site for 24 h. An occlusive patch containing the test substance was then applied to the same site for 48 h. The preceding patch application

TABLE 12
Case reports on Gum Arabic and other species of Acacia

Ingredient studied	Patients evaluated	Procedure/route of exposure	Results	Reference
Acacia	78-year-old male with hard nodular mass in right upper quadrant (shock symptoms reported)	Subcutaneous injection of two doses of the drug tyramin (0.06 g/dose). Second dose followed by i.v. dose of 6% Acacia in saline (500 cc)	Death accelerated by intravenous administration of Acacia solution	Lee 1922
Acacia	Male patient with pulmonary hemorrhage	Intravenous administration of 6% Acacia in saline (150 cc)	Patient's condition worsened immediately after injection, followed by death 2 h 20 min later	Lee 1922
Acacia	27-year-old female recovering from elephantiasis surgery	Intravenous administration of 6% Acacia solution (500 cc) and 500 cc of physiologic saline solution after initial surgery and after second operation 7 months later	No adverse effects after first infusion. Signs/symptoms noted after second infusion: nasal obstruction and lacrimation, followed by difficulty in breathing, coughing, and suggestion of laryngeal stridor. Symptoms disappeared rapidly after epinephrine administration	Maytum and Magath 1932
Acacia	15 kidney transplant patients. Itching/rash in 3 patients	Patients had been treated with prednisone and azathioprine for 10 months to 5 years. Prednisone tablets contained Acacia and tragacanth gums as adhesives. Itching/rash not observed after tablets withdrawn. Scratch tests performed	Scratch test results for 2 of 3 patients with reactions tested: Positive reactions to Acacia and tragacanth gums, respectively. Scratch test results negative in remaining transplant patients	Rubinger et al. 1978
Gum Arabic	65-year-old male with allergic reactions	Four allergic accidents experienced after drinking coffee. Gum arabic used to coat roasted coffee beans. Prick tests and human basophil degranulation tests performed	Dual sensitization to coffee and gum arabic	Moneret-Vautrin 1993
Gum Arabic	57-year-old male with chronic alveolitis	Chronic alveolitis due to repeated and prolonged inhalation of sweets containing gum arabic.	Progress satisfactory in terms of clinical status and lung function measurement after exposure discontinued	De Fenoyl et al. 1987

Acacia (crude and purified forms)	53-year-old plaster molder in candy factory with bronchial asthma	Bronchial asthma due to inhalation of dust from factory environment. Scratch and intradermal injection tests performed	Markedly positive reaction to crude Acacia. Purified Acacia more reactive; induced positive reactions when tested at concentrations as low as 1:5000 dilution in scratch and intradermal injection tests	Spielman and Baldwin 1933
Gum Arabic (extracted with sodium carbohydrate buffer)	50-year-old confectioner at candy factory with strong respiratory allergy to gum arabic	Skin prick and intracutaneous tests	No reaction to gum arabic (1:1000 dilution) in prick test. Intracutaneous test results: +++ (1:10,000 dilution), + (1:100,000 dilution), and (1:1,000,000 dilution). Evaluation of IgE antibody response indicated that patient's serum reacted strongly to gum arabic	Fötisch et al. 1998
Gum Arabic	53-year-old printer with asthma	Asthma due to exposure to offset spray containing gum arabic. Repeat cutaneous and intracutaneous tests performed	4+ reaction to gum arabic in repeat cutaneous and intracutaneous tests	Bohner et al. 1941
Gum Acacia	32 male printers with asthma	Exposure to spray (used in color-printing) containing Gum Acacia and isopropyl alcohol. Average duration of exposure = 4 to 8 years	Asthma developed after exposure to spray	Fowler 1952
Gum Arabic	12 employees of gum processing factory (office and mill workers)	Sensitization test performed	Seven of 12 workers had positive skin reactions to gum arabic. All 12 had respiratory symptoms that were of an allergic nature	Gelfand 1943
Gum Arabic (as supplied)	24-year-old printer with 3-month history of hand dermatitis	Exposure to gum arabic on the job. Patch tests (Finn chambers) performed	++ reaction to gum arabic	Freeman 1984
Wet clay containing 5 to 7% gum arabic	45-year-old female with rash on hands	Exposure to wet clay for 2 years on the job. Patch tests performed	+ reaction to 1% and 5% aqueous gum arabic. ++ reaction to 25% aqueous gum arabic	Ilchyshyn and Smith 1985
Gum Arabic	44-year-old lithoprinter with 2-year history of hand eczema	Exposure to gum arabic (used to coat printing plates) on the job. Eczema worsened after exposure to gum arabic. Patch testing of 10% aqueous gum arabic	Positive patch test reaction to 10% aqueous gum arabic	van Ketel 1984

sequence was repeated for a total of five induction exposures, after which a 10-day nontreatment period was observed. Prior to challenge patch application, a new site on the opposite arm, forearm, or side of the back was pretreated for 1 h with 5% aqueous SLS (0.1 ml under occlusive patch). A single challenge patch (occlusive patch) was then applied to the same site for 48 h. Reactions were scored at 1 and 24 h after patch removal according to the following scale: 0 (not sensitized) to 3 (strong sensitization [large vesiculobullous reaction]).

Sensitization reactions were not observed at 1 or 24 h after challenge patch removal. It was concluded that, under the conditions of this test, the mascara containing 8.0% Acacia Senegal did not possess a detectable contact-sensitizing potential, and, hence, is not likely to cause contact sensitivity reactions under normal use conditions (Ivy Laboratories 2000).

Acacia (Not Gum Arabic)

Letizia et al. (2000) evaluated the sensitization potential of Acacia Farnesiana Extract (from flowers, 4% in petrolatum) in a maximization test using 30 healthy male and female volunteers. The test substance was applied, under occlusion, to the same site on both forearms of each subject throughout induction. The induction phase consisted of a total of five 48-h exposures (on alternate days).

Prior to application of the initial induction patch, the test site was pretreated with 5% aqueous SLS, under occlusion. The induction phase was followed by a 14-day nontreatment period, after which a challenge patch was applied (48 h) to new sites on each subject. Challenge patch applications were preceded by 30 min applications of 2% aqueous SLS, under occlusion, on the left side. Challenge sites on the right side were not pretreated. A fifth challenge site (petrolatum applied) served as the control.

It was concluded that none of the reactions observed could be classified as a significant skin irritation or allergic reaction (Letizia et al. 2000).

Case Reports

Gum Arabic

Gelfand (1949) reported allergic disorders in 10 subjects (7 males, 3 females; 11 to 55 years old) who had ingested various gum-containing foods. Gum arabic was among the gums present in each food ingested. Some of the allergic symptoms reported included bronchial asthma, generalized urticaria, and vasomotor rhinitis. Allergic symptoms were not observed upon removal of suspect gum-containing foods from the diet, and symptoms were reproduced when clinical trials were repeated.

Positive skin reactions (test procedure not stated) to gum arabic were observed in each of the 10 subjects. The results of serologic studies (sera from four subjects) indicated that gum arabic was the dominant gum antigen in two subjects and that tragacanth and karaya were the dominant gum antigens in the remaining two subjects. The serological studies included passive transfer tests in serial dilutions and neutralization studies. It was determined that gum arabic and other vegetable gums could cause allergic disorders by ingestion in sensitive subjects (Gelfand 1949).

Raghuprasad et al. (1980) reported cross-reactivity between Gum Acacia and gum tragacanth in a 24-year-old patient who developed sensitization to Quillaja bark (*Quillaja saponaria*) dust, which resulted in rhinitis and asthma. The CIR Expert Panel has previously evaluated the safety of Tragacanth Gum in cosmetics, and concluded that this ingredient is safe in the present practices of use and concentration (Elder 1987). Specific immunoglobulin E(IgE) to pulverized Quillaja bark, gum arabic, and gum tragacanth were measured according to a modification of the radioallergosorbent test (RAST). Each of the three antigens (20 mg/ml) was coupled directly to methyl cellulose disks that had been activated previously by cyanogen bromide dissolved in acetonitrile. Results were expressed as percent binding.

The amount of radioactivity bound by the patient's serum was compared with control sera from healthy, nonallergic volunteers (number not stated) not known to be exposed to Quillaja bark dust. The mean percent binding of IgE to Quillaja bark in patient sera was 22.4%, compared to 3.2% for the control. Compared to negligible binding in control sera, significant binding was reported for gum arabic (32.5% binding) and gum tragacanth (30.8% binding) (Raghuprasad et al. 1980).

Additional case reports on gum arabic and other species of *Acacia* are summarized in Table 12. Although two fatalities are reported, neither related to gum arabic and most case reports involve sensitization reactions.

SUMMARY

This safety assessment includes the following ingredients, derived from Acacia, that are listed in the *International Cosmetic Ingredient Dictionary and Handbook*: 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.

Gum arabic is another name for Acacia Senegal Gum. Gum arabic is generally recognized as safe for direct addition to food for human ingestion. Acacia Senegal Gum has been described as the major commercial Acacia gum. Gum arabic is produced when the Acacia tree is stressed by infection, poor nutrition, heat, or lack of moisture. The gum exudes through wounds in the bark that occur naturally or are purposely made to stimulate production.

Gum arabic is composed of D-galactose, L-rhamnose, L-arabinose, and D-glucuronic acid residues in an arrangement of a main chain of galactosyl units joined by β-D-(1 $\rightarrow$ 3) linkages and side chains or branched oligosaccharides linked to the main chain by β-D-(1 $\rightarrow$ 6) linkages. It has also been described as a complex mixture of calcium, magnesium, and potassium salts of arabic acid. Arabic acid is a complex of galactose, rhamnose, arabinose, and glucuronic acid.

Aflatoxin has been reported as an impurity in Acacia Catechu, but not in gum arabic. Acacia Gum (Acacia Senegal) did not contain detectable pesticide residues.

Qualitative information is available on the components that may be found in ingredients derived from various Acacia species and plant parts. This information indicates a few similarities and many differences in constituents. Quantitative or semiquantitative data were not available.

The principal UV absorbance of gum arabic occurred at wavelengths below 240 nm. At wavelengths above 240 nm, the UV absorbance was not significant.

Acacia Decurrens Extract and Acacia Farnesiana Extract are described as a cosmetic astringent, and Acacia Decurrens is also described as a skin-conditioning agent—occlusive, although none of these are reported to be in current use. Cosmetic functions of the other Acacia-derived ingredients included in this review are not described. Product formulation data submitted to the FDA indicated that Acacia was used in 22 cosmetic products—no information is available to further describe the species or plant part. Cosmetic use concentration data supplied by the cosmetics industry indicated maximum use concentrations of 9% in shampoos for Acacia Senegal Gum and 0.001% for Acacia Senegal Gum Extract, in bath soaps and detergents.

Recommended use concentrations of Acacia Concinna Fruit Extract are 1.0% to 2.0% for use in shampoos, hair packs, hair conditioners, and hair rinses, although no uses have been reported to FDA.

The weight gain for rats fed gum arabic at a dietary concentration of 16% was 75% of that reported for control rats. Approximately 80% of the gum arabic was absorbed. Results from other studies involving rats suggest that the metabolism of gum arabic is mediated by bacteria in the cecum.

Results of studies in which dogs and rabbits were injected intravenously with gum arabic indicated that gum arabic or some other product associated with it accumulated in the liver and remained in the tissues for several months. Nonlethal effects included disturbances in hemoglobin values, white blood cells, and serum proteins.

Based on absorption and metabolism studies an expert analysis determined that gum arabic is capable of being digested to simple sugars. It was also determined that conclusive evidence indicating that the intact gum arabic molecule is absorbed under normal conditions was lacking.

In an in vitro assay, dose-dependent uncoupling of oxidative phosphorylation was noted in groups of rats dosed orally with gum arabic up to 10% twice daily for 4 weeks, but comparable biochemical effects were not observed in vivo.

An acute oral LD_{50} of 8000 mg/kg was reported for Acacia Gum in rabbits. The acute oral LD_{50} for Acacia Farnesiana Extract (from flowers) in rabbits was >5.0 g/kg. A minimal lethal dose of >2 g/kg (10 ml/kg) for Acacia Dealbata Leaf Wax in a suspension with paraffin oil was reported in an acute oral toxicity study involving rats. None of the animals died, and no test substance-related lesions of organs examined were noted at necropsy. Similarly, in another study, no deaths or test substance-related, organ lesions were reported following the oral administration of Acacia Farnesiana Flower Wax at a dose of 10 ml/kg.

In an acute dermal toxicity study of Acacia Farnesiana Extract (from flowers) involving rabbits, an LD_{50} of >5.0 g/kg was reported.

Gum arabic did not cause any abnormal changes in serum chemistry parameters or induce toxicologically significant lesions in rats that received oral doses daily for 28 days. Gum arabic was also administered to rats in four other short-term oral toxicity studies. Collectively, test concentrations ranged from 1% to 20% and study durations ranged from 28 days to 9 weeks. No significant or discernible ultrastructural differences were found between tissues (heart, liver, small intestine) of control rats and test rats; hematological findings were normal. Gum arabic was nontoxic, even at the highest concentration tested.

One of three dogs injected intravenously (32 to 35 injections) with gum arabic over a period of 76 days died. The range for the total cumulative dose was 15.7 to 47.7 g/kg, and death occurred at the highest dose (47.7 g/kg). An enlarged liver was observed in the animal that died, and the cause of death was not determined. Enlarged livers and swollen kidneys were also observed in dogs that received doses ranging from 1 to 2 g/kg.

In a subchronic (13 weeks) oral toxicity study on Acacia Senegal Gum, the only treatment-related alteration noted in rats at necropsy was cecal enlargement in animals of the highest dose (14 g/kg/day) groups.

Electron microscopic findings for samples of livers and kidneys from groups of five rats fed diets containing 0.5% to 3.5% w/w Acacia Senegal Gum daily for 91 days were negative. Mitochondria and nuclei were ultrastructurally normal in appearance and internal structure.

The administration of a single dermal dose of Acacia Farnesiana Extract (from flowers, 5.0 g/kg) to rabbits induced moderate erythema and edema. Undiluted Acacia Dealbata Leaf Wax was classified as a non-irritant after application, under occlusive patches, to scarified skin of albino rabbits for 24 h. Undiluted Acacia Farnesiana Flower Wax was classified as a slight irritant when tested according to a similar procedure.

A 20.0% solution of Acacia Farnesiana Extract in methanol (from flowers) did not induce phototoxicity in SKH:hairless mice.

Anaphylactic signs in guinea pigs injected intraperitoneally (mild challenge reactions) or intravenously (strong challenge reactions) with Acacia solution have been reported. No signs of anaphylaxis were observed in rabbits injected intravenously (no challenge reaction) with Acacia solution. In rabbits and guinea pigs injected with 7% Gum Acacia solution, no deleterious effects on antibody production resulted.

Mouse footpad swelling test results indicated a significant increase in footpad thickness (compared to controls) in mice immunized by injection of gum arabic in saline and Freund's adjuvant. Antigen-specific hypersensitivity reactions were noted. In a similar test, footpad swelling was significantly suppressed

(compared to controls) in mice dosed orally with gum arabic and then immunized by injection of gum arabic in saline and Freund's adjuvant. In another test, intradermal challenge after immunization of mice with Acacia Senegal Gum caused a significant increase in footpad thickness.

Gum arabic was not mutagenic in numerous in vitro mutagenicity tests using *Salmonella typhimurium, Saccharomyces cerevisiae,* and *Bacillus subtilis* bacterial strains. In an in vitro cytogenetics assay, though results were classified as slightly positive, gum arabic did not induce definite abnormal anaphase figures in diploid human embryonic lung (WI-38) fibroblasts. The mutagenicity of gum arabic was also evaluated in numerous in vivo assays, the results of which were mostly negative.

Statistically significant positive results were noted in one of the three dominant lethal tests (rat assay, but not in two mouse assays) that were performed. Further testing in the mouse heritable translocation test yielded negative results. In acute and short-term in vivo cytogenetics assays (rats), though no significant positive responses were observed, there may have been a slight positive response. It was stated that further tests and a detailed statistical evaluation are needed in order to confirm this possibility. There were no statistically significant findings in mouse chromosomal aberrations and sperm-head morphology assays. Negative results were also reported in micronucleus tests (mouse bone marrow smears) and other in vivo assays.

No evidence of carcinogenicity was observed in rats dosed intraperitoneally with gum arabic (1.75% or 7.0% in saline or water) three times per week for up to 15 weeks. In another study, tumors were not observed in guinea pigs injected intramediastinally with 0.1 ml of a gruel of gum arabic (single dose).

The carcinogenicity of gum arabic was also evaluated using 4-week-old F344 and 4- to 5-week-old $B6C3F_1$ mice. Low-dose animals were fed gum arabic at a concentration of 25 g/kg in the diet and high-dose animals were fed 50 g/kg for 103 weeks. Neoplasms were observed only in male rats, and were diagnosed as malignant lymphomas or leukemia-lymphoma. Compared to controls, no significant increases were observed in the incidence of either type of neoplasm at either of the two test concentrations; gum arabic was classified as noncarcinogenic in rats and mice.

Oral administration of gum arabic (1 ml) did not cause antifertility effects in female rats or the suppression of spermatogenesis in male rats. Gum arabic was not teratogenic when administered orally to mice at doses up to 1600 mg/kg. Oral doses of gum arabic up to 1600 mg/kg also were not teratogenic in rats and hamsters, and oral doses up to 800 mg/kg were not teratogenic in rabbits.

No effects on fertility or ovulation (4% gum arabic), or any abnormal variations in blastocysts (10% gum arabic) were found in rabbits. Gum arabic, at a concentration of 15%, failed to induce teratogenicity or other reproductive effects in female rats. Gum arabic (5%) also did not cause abnormal sperm development in hamsters. Embryotoxicity was not noted in mice injected intraperitoneally with a 1% aqueous suspension or mucilage prepared from gum arabic.

No evidence of absorption of intact gum arabic was found in 22 infants fed gum arabic in milk. In a patient with nephrosis, 20% of the gum arabic injected intravenously was excreted in the urine over a period of 6 weeks. Gum arabic was not detected in feces specimens collected from five male volunteers before or after administration of the gum.

Toxic effects were not observed in five male subjects who ingested 25 g of gum arabic daily for 21 days.

In a 48-h closed patch test, Acacia Farnesiana Extract (from flowers, 4.0% in petrolatum) did not induce skin irritation in any of the 30 subjects tested. In an "in-use test," skin irritation was not observed in any of the 30 subjects tested with Acacia Concinna Fruit Extract (2% in natural base [such as carageenan] and a routine shampoo base). The test substance remained in contact with the scalp for 10 to 15 min, and skin irritation was evaluated immediately after application and 24 and 48 h later.

The skin sensitization potential of a mascara containing 8.0% Acacia Senegal was evaluated in the maximization test using 28 healthy adult volunteers. It was concluded that, under the conditions of this test, the mascara containing 8.0% Acacia Senegal did not possess a detectable contact-sensitizing potential, and, hence, is not likely to cause contact sensitivity reactions under normal use conditions.

The results of a study involving ten subjects who had ingested various gum-containing foods, indicated that gum arabic could cause allergic disorders in sensitive subjects. Analyses of sera from 4 of the 10 subjects indicated that gum arabic was the dominant gum antigen in two subjects. Cross-reactivity between gum arabic and gum tragacanth was reported for a 24-year-old patient who developed sensitization to Quillaja bark (*Quillaja saponaria*) dust, which led to rhinitis and asthma.

Neither significant skin irritation nor allergic reactions to 4% Acacia Farnesiana Extract (from flowers) in petrolatum were observed in a maximization test (30 subjects).

A number of case reports of gum arabic allergenicity have been identified in the published literature.

DISCUSSION

Extensive safety test data are available on gum arabic that demonstrate its safety in a wide variety of applications, including cosmetic use. Based on the available information, the Panel concluded that Acacia Senegal Gum is equivalent to gum arabic and should be considered safe as used in cosmetics. It also appears that gums from other species are not the same as Acacia Senegal Gum. It follows that the safety test data on gum arabic can be used to support the safety of Acacia Senegal Gum and not gum from other Acacia species. Because Acacia Senegal Gum Extract is derived from Acacia Senegal Gum, the Panel considered that Acacia Senegal Gum Extract would present no additional safety issues.

The Panel recognized the potential for allergic responses to gum arabic. However, because of negative results for all 25 subjects in a human maximization study (mascara containing 8%

Acacia Senegal) and the expected slow rate of dermal absorption of gum arabic due to its large molecular size and water solubility, the Panel determined that it is not likely that normal use of gum arabic in a cosmetic product would result in sensitization.

The Panel is concerned that the available data suggesting the absence of pesticide residues in Acacia plants harvested wild are limited. The Panel advised the industry that the total polychlorinated biphenyl (PCB)/pesticide contamination of any plant-derived cosmetic ingredient should be limited to not more than 40 ppm, with not more than 10 ppm for any specific residue. The Panel also advised that limits were appropriate for the following impurities: arsenic (3 mg/kg maximum), heavy metals (0.002% maximum), and lead (5 mg/kg maximum).

The limited safety test data on Acacia Farnesiana Extract and on Acacia Concinna Fruit Extract were not sufficient to assess the safety of these ingredients in cosmetics.

The Panel found no information that adequately characterized the composition of fruit, leaf or other extracts, leaf wax, flower wax, or gum from Acacia species other than *A. senegal*. Therefore, the Panel could not extrapolate the available data on gum arabic to support the safety 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, and Acacia Senegal Extract. The Panel concluded that available data are insufficient to support the safety of these Acacia-derived ingredients.

The additional data needed for these ingredients include

1. concentration of use in cosmetics;
2. identify the chemical composition; if they are sufficiently different from those of Acacia Senegal Gum, then the following data would be needed:
   a. UV absorption spectrum; if there is significant absorbance in the UVA or UVB range, then phototoxicity and photosensitization studies may be needed;
   b. with the exception of Acacia Farnesiana Extract and Acacia Concinna Fruit Extract, sensitization and irritation data are needed;
   c. two genotoxicity assays, one in a mammalian system; if positive, then a 2-year dermal carcinogenicity study using National Toxicology Program (NTP) methods may be needed;
   d. dermal absorption data; if there is any evidence of significant dermal absorption, then reproductive and developmental toxicity data may be needed.

CONCLUSION

Based on the available animal and clinical data included in this report, the CIR Expert Panel concluded that Acacia Senegal Gum and Acacia Senegal Gum Extract are safe as used in cosmetic products. The Panel also concluded that the available data are insufficient to support the safety of the following ingredients in cosmetic products: 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, and Acacia Senegal Extract.

REFERENCES

Abdalla, M. H. 1988. Isolation of aflatoxin from *Acacia* and the incidence of *Aspergillus flavus* in the Sudan. *Mypathologia* 104:143–148.

Abul Gafar, M., T. Obata, F. Kiuchi, and Y. Tsuda. 1997. Acacia concinna saponins. I. Structures of prospogenols, concinnosides A-F, isolated from the alkaline hydrolysate of the highly polar saponin fraction. *Chem. Pharm. Bull. (Tokyo)* 45:620–625.

Akbarsha, M. A., and B. Manivannan. 1973. Biochemical changes in the testis and male accessory organs of albino rats on treatment with *Andrographis paniculata* (NEES). *Ind. J. Comp. Anim. Physiol.* 11:103–108.

Ames, B. N. 1971. The detection of chemical mutagens with enteric bacteria, pp. 267–282. In A. Hollaender (ed.), *Chemical Mutagens, Principles & Methods for Their Detection*. Plenum Press, New York, NY.

Ames, B. N., J. McCann, and E. Yamasaki. 1975. Methods of detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. *Mutat. Res.* 31:347–364.

Anderson, D. M. W. 1986. Evidence for the safety of gum arabic (*Acacia senegal (L.)* Wild.) as a food additive—a brief review. *Food Addit. Contam.* 3:225–230.

Anderson, D. M. W. 1988. The natural gums. *Bri. Nutr. Found. Nutr. Bull.* 13:101–113.

Anderson, D. M. W., P. Ashby, A. Busuttil, M. A. Eastwood, B. M. Hobson, A. H. Ross, and C. A. Street. 1982. Subchronic effects of gum arabic (*Acacia*) in the rat. *Toxicol. Lett.* 14:221–227.

Anderson, D. M. W., P. Ashby, A. Busuttil, S. A. Kempson, and M. E. Lawson. 1984. Transmission electron microscopy of heart and liver tissues from rats fed with gums arabic and tragacanth. *Toxicol. Lett.* 21:83–89.

Anderson, D. M. W., A. Busuttil, S. A. Kempson, and D. W. Penman. 1986. Transmission electron microscopy of jejunum, ileum, and cecum tissues from rats fed with gums arabic, karaya and tragacanth. *Toxicology* 41:75–82.

Anderson, D. M. W., D. M. B. Douglas, N. A. Morrison, and W. Weiping. 1990. Specifications for gum arabic (*Acacia senegal*); analytical data for samples collected between 1904 and 1989. *Food Addit. Contam.* 7:303–322.

Anderson, D. M. W., J. R. A. Millar, and W. Weiping. 1991. Gum arabic (*Acacia senegal*): Unambiguous identification by ^{13}C-NMR spectroscopy as an adjunct to the Revised JECFA Specification, and the application of ^{13}C-NMR spectra for regulatory/legislative purposes. *Food Addit. Contam.* 8:405–422.

Anonymous. 1993. Twenty Australian *Acacias* and the doctors whose lives and works they commemorate. *Med. J. Aust.* 159:731–738.

Aronson, S. B., and R. B. McMaster. 1972. Mechanism of the host response in the eye. VI. Immune and toxic stimulation of inflammation in the germ-free guinea pig. *Arch. Ophthalmol.* 88:533–539.

Avon Products, Inc. 2000a. UV absorption of *Acacia senegal*, Avon Code RI-0253. Analytical laboratory report (Analytical job number 69669). Unpublished data submitted by CTFA, March 27, 2000. (2 pages.)[2]

Avon Products, Inc. 2000b. UV absorption of acacia senegal, Avon Code RI-0253. Analytical laboratory report (Analytical job number 70384). Unpublished data submitted by CTFA, March 27, 2000. (2 pages.)[2]

Bachmann, E., E. Weber, M. Post, and G. Zbinden. 1978. Biochemical effects of gum arabic, gum tragacanth, methylcellulose, and carboxymethylcellulose-Na in rat heart and liver. *Pharmacology* 17:39–49.

Bertin Laboratories. 1987. Study of primary cutaneous tolerance of the product Cassia Essential Wax [Acacia Farnesiana Flower Wax] (Study No. F-950). English translation. Unpublished data submitted by CTFA, February 14, 2001. (11 pages.)[2]

[2]Available for review from the Director, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 310, Washington, DC 20036, USA

Biogir S. A. Conseil Recherche. 1990a. Assessment of acute toxicity in rat. Cire Essentielle De Fleurs De Mimosa [Acacia Dealbata Leaf Wax] (Study No. TAO 90.786). Unpublished data submitted by CTFA, February 14, 2001. (15 pages.)[2]

Biogir S. A. Conseil Recherche. 1990b. Assessment of cutaneous tolerance in rabbit. Index of primary cutaneous irritation. Cire Essentielle De Fleurs De Mimosa [Acacia Dealbata Leaf Wax] (Report No. IC 90.786). Unpublished data submitted by CTFA, February 14, 2001. (13 pages.)[2]

Bohner, C. B., J. M. Sheldon, and J. W. Trenis. 1941. Sensitivity to gum acacia, with a report of ten cases of asthma in printers. *J. Allerg.* 12:290–294.

Brusick, D. J., and V. W. Mayer. 1973. New developments in mutagenicity screening techniques using yeast. *Environ. Health Perspect.* 6:83–96.

Carlisle International Corporation. 1997a. Additional data for Acacia Concinna. First Submission. Unpublished data submitted by Carlisle International Corporation, September, 1997. (1 page.)[2]

Carlisle International Corporation. 1997b. Additional data for Acacia Concinna. Second Submission. Unpublished data submitted by Carlisle International Corporation, December, 1997. (6 pages.)[2]

Chaudhari, P. N., and V. G. Hatwalne. 1973. Effect of katha (*Acacia catechu*) on serum and liver cholesterol levels in rats. *Indian J. Nutr. Dietet.* 10:130–133.

Collins, T. F. X., J. J. Welsh, T. N. Black, S. L. Graham, and L. H. Brown. 1987. Study of the teratogenic potential of gum arabic. *Food Chem. Toxicolo.* 25:815–821.

Cook, W. M., R. Purchase, G. P. Ford, D. M. Creasy, P. G. Brantom, and D. Gangolli. 1992. A 28-day feeding study with ethyl acetoacetate in rats. *Food Chem. Toxicolo.* 30:567–573.

CTFA. 2000a. Use data collected by CTFA in 1999. Unpublished data submitted by CTFA. (1 page.)[2]

CTFA. 2000b. Unpublished data submitted by CTFA. (1 page.)[2]

Dangerous Properties of Industrial Materials Report. 1981. Hazardous Materials Section. *Dangerous Properties of Industrial Materials Report* 1:17–87.

De Fenoyl, O., F. Capron, F. Guyon, B. Lebeau, and J. Rochemaure. 1987. Inhalation pneumonia presenting as a pneumocystitis infection. *Rev. Mal. Respir.* 4:97–100.

Elder, R. L., ed. 1987. Final report on the safety assessment of tragacanth gum. *J. Am. Coll. Toxicol.* 6:1–22.

El-Hamid, A., and I. Sidrak. 1970. The investigation of *Acacia farnesiana* essential oil. *Planta Med.* 18:98–100.

European Economic Community (EEC). 2001. EEC Cosmetics Directive 76/768/EEC, as amended, Annexes I through VII. Brussels: EEC.

European Federation for Cosmetic Ingredients (EF*f*CI). 2000. Unpublished data on Acacia catechu, Acacia concinna, Acacia decurrens var. dealbata, Acacia decurrens, Acacia farnesiana, Acacia arabica=Acacia nilotica, Acacia mearnsii, Acacia melanoxylon, Acacia nilotica, Acacia senegal, Arabic gum, and Acacia ssp. Submitted by the EF*f*CI.[2]

Federation of American Societies for Experimental Biology (FASEB). 1973. Evaluation of the health aspects of gum arabic as a food ingredient. NTIS Report No. PB-234 904.

Food and Agriculture Organization of the United Nations (FAO). 1990. Specifications for identity and purity. *Food Nutr. Paper* 49:23–25.

Food and Agriculture Organization of the United Nations (FAO). 1999. *Compendium of food additive specifications. Addendum 7* (Joint FAO/World Health Organization Expert Committee on Food Additives. 53rd Session), 1–2 Rome: FAO.

Food Chemicals Codex. 1996. Specifications on acacia. *Food Chemicals Codex*, 4th ed., 9. Washington, DC: National Academy Press.

Food and Drug Administration (FDA). 2001. Frequency of use of cosmetic ingredients. *FDA Database*. Washington, DC: FDA.

Food and Drug Research Laboratories. 1972. Teratologic evaluation of FDA 71–75 (Gum Arabic). NTIS Report No. PB-221 796.

Fötisch, K., J. Fäh, B. Wüthrich, F. Altmann, D. Haustein, and S. Vieths. 1998. IgE antibodies specific for carbohydrates in a patient allergic to gum arabic (*Acacia senegal*). *Allergy* 53:1043–1051.

Fowler, P. B. S. 1952. Printer's asthma. *Lancet* 2:755–757.

Freeman, S. 1984. Allergic contact dermatitis due to 1,2-benzisothiazolin-3-one in gum arabic. *Contact Dermatitis* 11:146–149.

Frohberg, H., H. Oettel, and H. Zeller. 1969. Mechanism of the teratogenic effect of tragacanth. *Arch. Toxicol.* 25:268–295.

Frutarom Meer Corporation. No date. Product information on gum arabic. Unpublished data submitted by CTFA, March 27, 2000. (2 pages.)[2]

Gabridge, M. G., and M. S. Legator. 1969. A host-mediated microbial assay for the detection of mutagenic compounds. *Proc. Soc. Exp. Biol. Med.* 130:831.

Gelfand, H. H. 1943. The allergenic properties of the vegetable gums. *J. Allergy* 14:203–219.

Gelfand, H. H. 1949. The vegetable gums by ingestion in the etiology of allergic disorders. *J. Allergy* 20:311–321.

Gennaro, A. R., ed. 1990. *Remington's Pharmaceutical Sciences*, 18th ed., 1304. Easton, PA: Mack Publishing Company.

Gottschalck, T. A., and G. N. McEwen, Jr., eds. 2000. *International Cosmetic Ingredient Dictionary and Handbook*, 10th ed. Washington, DC: CTFA.

Green, S. 1977. Present and future uses of mutagenicity tests for assessment of the safety of food additives. *J. Environ. Pathol. Toxicol.* 1:49–54.

Hagos, M., and G. Samuelsson. 1988. Quantitative determination of quracol A, B and (+)-fisetinidol in bark and gum of *Acacia tortilis*. *Acta Pharm. Suec.* 25:321–324.

Haworth, S., T. Lawlor, K. Mortelmans, W. Speck, and E. Zeiger. 1983. *Salmonella* mutagenicity results for 250 chemicals. *Environ. Mutagen* 5:3–142.

Heidelberger, M., O. T. Avery, and W. F. Goebel. 1929. A soluble specific substance derived from gum arabic. *J. Exp. Med.* 49:847–857.

Huynh, P. N., A. P. S. Hikim, C. Wang, K. Stefonovic, Y. H. Lue, A. Leung, V. Atienza, S. Baravarian, V. Reutrakul, and R. S. Swerdloff. 2000. Long-term effects of triptolide on spermatogenesis, epididymal sperm function, and fertility in male rats. *J. Androl.* 21:689–699.

Ilchyshyn, A., and G. Smith. 1985. Gum arabic sensitivity associated with epidemic hysteria dermatological. *Contact Dermatitis* 13:282–283.

Informatics Inc. 1972. GRAS (Generally Recognized as Safe) food ingredients—gum arabic. NTIS Report No. PB 223 614.

Ishizaki, M., and S. Ueno. 1987. The DNA-damaging activity of natural food additives (IV). *Shokuhin Eiseugaku Zasshi (J. Food Hyg. Soc. Japan)* 28:498–501.

Ivy Laboratories. 2000. An evaluation of the contact-sensitization potential of a topical coded product (mascara containing 8% Acacia Senegal) in human skin by means of the maximization assay. Unpublished data submitted by CTFA, March 27, 2000. (11 pages.)[2]

Jain, A. K., K. Shimoi, Y. Nakamura, I. Tomita, and T. Kada. 1987. Preliminary study on the desmutagenic and antimutagenic effect of some natural products. *Curr. Sci.* 57:1266–1269.

Joint FAO/WHO Expert Committee on Food Additives (JECFA). 1990. Evaluation of certain food additives & contaminants. 35th Report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, No. 789.

Joshi, K. C., M. K. Tholia, and T. Sharma. 1975. Chemical examination of *Acacia modesta*. *Planta Med.* 27:281–283.

Kar, R. N., K. Khan, and S. K. Mukherjee. 1984 *In vivo* mutagenic effect of methyldopa. 1. Dominant lethal test in male mice. *Cytobios* 41:163–164.

Katz, M., J. A. Heddle, and M. F. Salamone. 1981. Mutagenic activity of polycyclic aromatic hydrocarbons and other environmental pollutants. In: a *Polynuclear Aromatic Hydrocarbons: Chemical Analysis and Biological Fate*, ed. M. Cooke and A. J. Dennis, 519–528. Columbus, OH: Battelle Press.

Lawrence, J. F., and J. R. Iyengar. 1985. Gas chromatographic determination of polysaccharide gums in foods after hydrolysis and derivatization. *J. Chromatogr.* 350:237–244.

Lee, R. V. A. 1922. Sudden death in two patients following intravenous injections of acacia. *JAMA* 26:726–727.

Letizia C. S., J. Cocchiara, G. A. Wellington, C. Funk, and A. M. Api. 2000. Monographs on Fragrance Raw Materials. Cassie Absolute (Acacia Farnesiana (L.) Willd.). *Food Chem. Toxicol.* 38:S27–S29.

Lewis, R. J., ed. 1993a. *Hawley's Condensed Chemical Dictionary*, 12th ed., 93. New York: Van Nostrand Reinhold.

Lewis, R. J., ed. 1993b. *Hazardous Chemicals Desk Reference*, 3rd ed., 87. New York: Van Nostrand Reinhold.

Litton Bionetics, Inc. 1975. Mutagenic evaluation of compound. FDA 71-15 PM9000-01-5, gum arabic. NTIS Report No. PB-267 348.

Lutz, W. K., E. Brandle, and G. Zbinden. 1978. Effect of gum arabic on aminopyrine demethylation in rats. *Experentia* 34:1609–1610.

MacGregor, J. T., C. M. Wehr, G. D. Manners, L. Jurd, J. L. Minkler, and A. V. Carrano. 1983. *In vivo* exposure to plant flavonols. Influence on frequencies of micronuclei in mouse erythrocytes and sister-chromatid exchange in rabbit erythrocytes. *Mutat. Res.* 124:255–270.

Marakesh, I., Y. Y. Mei, M. Go, and J. Haginawa. 1969. Isolation of (−)-trans-4-hydroxypipecolic acid from the young leaves of *Acacia mollissima*. *Yakugaku Zasshi* 89:1723–1725.

Marrack, J., and B. R. Carpenter. 1938. The cross reactions of vegetable gums with type II antipneumococcal serum. *Br. J. Exp. Pathol.* 19:53–65.

Matsuzawa, S. 1968. Immunochemical studies on gum arabic. 4) Blood group antigens other than Le^a in gum arabic. *Nippon Hoigaku Zasshi* 22:283–289.

Maxwell, W. A., and G. W. Newell. 1974. Screening techniques for environmental mutagens. In *Molecular & Environmental Aspects of Mutagenesis*, 6th Rochester International Conference on Environmental Toxicity (1973). Louise Prakash, ed. Ch 14, pp. 223–252.

Maytum, C. K., and T. B. Magath. 1932. Sensitivity to acacia. *JAMA* 99:2251.

Melnick, R. L., J. Huff, J. K. Haseman, M. P. Dieter, C. K. Grieshaber, D. S. Wy, A. B. Russfield, A. S. K. Murthy, R. W. Fleischman, and H. S. Lilja. 1983. Chronic effects of agar, guar gum, gum arabic, locust-bean gum, or tara gum. *Food Chem. Toxicol.* 21:305–311.

Ministry of Health, Labor and Welfare (MHLW). 2000a. Unofficial translation of MHW Ordinance No. 331, Attached Table 1 [Negative List]. Ministry of Health Labor and Welfare, Pharmaceutical and Medical Safety Bureau, Inspection and Guidance Division, 2-2,1-chome, Kasumigaseki, Chiyoda-ku, Tokyo 100-8045, Japan.

MHLW. 2000b. Unofficial translation of MHW Ordinance No. 331, Attached Table 2 [Restricted List]. Ministry of Health Labor and Welfare, Pharmaceutical and Medical Safety Bureau, Inspection and Guidance Division, 2-2,1-chome, Kasumigaseki, Chiyoda-ku, Tokyo 100-8045, Japan.

Moneret-Vautrin, D. A., G. Kanny, J. P. Faller, D. Levan, and C. Kohler. 1993. Severe anaphylactic shock with heart arrest caused by coffee and gum arabic, potentiated by beta-blocking eyedrops. *Rev. Med. Interne* 14:107–111.

Morseth, S. L., and T. Ihara. 1989a. Teratology study in rats with manidipine hydrochloride. *Yakuri To Chiryo* 17:163–183.

Morseth, S. L., and T. Ihara. 1989b. Reproduction study in rats with manidipine chloride. *Yakuri To Chiryo* 17:145–162.

Narita, K. 1985. Immunogenic specificity of gum arabic and genetic regulation of $Lewis^a$ expression. *Nippon Hoigaku Zasshi* 39:275–290.

Neal, S. B., and G. S. Probst. 1983. Chemically-induced sister-chromatid exchange in vivo in bone marrow of Chinese hamsters. An evaluation of 24 compounds. *Mutat. Res.* 113:33–43.

Nichols, W. W., P. Moorhead, and G. Brewen, 1971. Chromosome methodologies in mutation testing. *Newslett. Environ. Mutagen Soc.* 5:20.

Oelrichs, P. B., and T. McEwan. 1962. The toxic principle of *Acacia georginae*. *Queensland J. Agr. Sci.* 19:1–16.

Ojha, D., G. Singh, and Y. N. Upadhyaya. 1969. Clinical evaluation of *Acacia catechu*, Wild (Khadira) in the treatment of lepromatous leprosy. *Int. J. Lepr. Other Mycobat. Dis.* 37:302–307.

Osswald, H. 1968. Influence of various polysaccharides on the growth behavior of the Ehrlich ascites tumor. *Arzneimittelforschung* 18:1495–1498.

Parton, J. W., G. S. Probst, and M. L. Garriott. 1988. The *in vivo* effect of 2,6-xylidine on induction of micronuclei in mouse bone marrow cells. *Mutat. Res.* 206:281–283.

Parton, J. W., J. E. Beyers, M. L. Garriott, and R. N. Tamura. 1990. The evaluation of a multiple dosing protocol for the mouse bone-marrow micronucleus assay using benzidine and 2,6-xylidine. *Mutat. Res.* 234:165–168.

Partridge, S. M., and W. T. J. Morgan. 1942. Artificial antigens with agar, gum acacia, and cherry gum specificity. *Br. J. Exp. Pathol.* 23:84–94.

Pazur, J. H., S. A. Kelly-Delcourt, F. J. Miskiel, L. Burdett, and J. J. Docherty. 1986. The isolation of anti-gum arabic antibodies by affinity chromatography. *J. Immunol. Methods* 89:19–25.

Pentiah, P. R., P. P. Reddy, and O. S. Reddi. 1989. Induction of micronuclei in bone marrow cells of mice treated with phenylbutazone. *Indian J. Exp. Biol.* 18:869–871.

Prasad, M. H., K. Pushpavathi, P. Rita, and P. P. Reddy. 1987. The effect of thiram on the germ cells of male mice. *Food Chem. Toxicol.* 25:709–710.

Prival, M. J., V. F. Simmon, and K. E. Mortelmans. 1991. Bacterial mutagenicity testing of 49 food ingredients gives very few positive results. *Mutat. Res.* 26:321–329.

Quereshi, M. Y., D. J. Pilbeam, C. S. Evans, and A. Bell. 1977. The neurolathyrogen, α-amino-β-oxalylaminopropionic acid in legume seeds. *Phytochemistry* 16:477–480.

Raghuprasad, M. D., S. M. Brooks, A. Litwin, J. J. Edwards, I. L. Bernstein, and J. Gallagher. 1980. Quillaja bark (soapbark)-induced asthma. *J. Allergy Clin. Immunol.* 65:285–287.

Rice, C. E. 1954a. Relative effects of various agents on complement and antibody production. *J. Immunol.* 73:375–382.

Rice, C. E. 1954b. An investigation of some of the factors determining the decrease in complement activity in anaphylactic shock. *J. Immunol.* 75:85–95.

Ross, A. H. M., M. A. Eastwood, J. R. Anderson, and D. M. W. Anderson, 1984a. A study of the effect of dietary gum arabic in humans. *Am J. Clin. Nutr.* 37:368–375.

Ross, A. H. M., M. A. Eastwood, W. G. Brydon, A. Busuttil, L. F. McKay, and D. M. W. Anderson. 1984b. A study of the effects of dietary effects of gum arabic in the rat. *Br. J. Nutr.* 51:47–56.

Roy, A. K., and V. Kumari. 1991. Aflatoxin and citrinin in seeds of some medicinal plants under storage. *Int. J. Pharmacogn* 29:62–65.

Roy, A. K., K. K. Sinha, and H. K. Chourasia, 1988. Aflatoxin contamination of some common drug plants. *Appl. Environ. Microbiol.* 54:842–843.

Rubinger, D., M. Friedlander, and E. Superstine. 1978. Hypersensitivity to tablet additives in transplant recipients on prednisone. *Lancet* 2:689.

Sabir, M., and M. K. Razdan. 1970. Antifertility study with leaf extract of *Adina cordifolia* (Karam Ki Gaach). *Indian J. Physiol. Pharmacol.* 14:209–210.

Schardein, J. L., T. Woosley, L. E. Hamilton, and D. H. Kaump. 1965. Effects of aspirin and phenylbutazone on the rabbit blastocyst. *J. Reprod. Fertil.* 10:129–132.

Secor, J. B., E. E. Conn, J. E. Dunn, and D. S. Seigler. 1976. Detection and identification of cyanogenic glucosides in six species of Acacia. *Phytochemistry* 15:1703–1706.

Seigler, D. S., J. E. Dunn, E. E. Conn, and J. F. Pereira. 1983. Cyanogenic glycosides from four Latin American species of *Acacia. Biochem. Syst. Ecol.* 11:15–16.

Sethi, N., R. K. Singh, and R. K. Srivastava. 1989. Embryotoxicity of synthetic steroid RU 38486. *Biol. Mem.* 15:107–110.

Shaligram, S. G., and J. R. Vakil. 1990. Report on dermocosmetological study by in-use testing of Folicon 100: Shikakai Extract (Acacia Concinna) in cosmetic formulations. Unpublished data submitted by CTFA, October 19, 2000. (9 pages.)[2]

Sham, J. S., K. W. Chiu, and P. K. T. Pang. 1984. Hypotensive action of *Acacia catechu*. *Planta Med.* 50:177–180.

Sheu, C. W., K. T. Cain, C. J. Rushbrook, T. A. Jorgenson, and W. M. Generoso. 1986. Tests for mutagenic effects of ammoniated glycyrrhizin, butylated hydroxytoluene, and gum arabic in rodent germ cells. *Environ. Mutagen* 8:357–368.

Silvette, H., O. Swineford, and L. Tull. 1955. Observations on acacia as an immunizing, sensitizing anaphylactogenic and desensitizing antigen. *J. Allergy* 26:509–518.

Smith, C. J., P. A. Williams, M. Jones, and G. O. Phillips. 1990. Procedure for detection of aflatoxins in gum arabic samples. *Food Hydrocolloids* 4:221–225.

Societe Bertin. 1987. Research on the acute toxicity in rats (using the oral method) of the product: Cire Essentielle Cassie [Acacia Farnesiana Flower Wax] (Study No. F 949/5304). English translation. Unpublished data submitted by CTFA, February 14, 2001. (16 pages.)[2]

Sotohy, S. A., W. Muller, and A. A. Ismail. 1995. "In vitro" effect of Egyptian tannin-containing plants and their extracts on the survival of pathogenic bacteria. *Dtsch. Tierarztl. Wochenschr.* 102:344–348.

Spielman, A. D., and H. S. Baldwin. 1933. Atopy to acacia (gum arabic). *JAMA* 101:444–445.

SRI International. 1980. Microbial mutagenesis testing of substances. Compound report: F76-069, gum arabic, Stein-Hall Co., Lot No. 55-3701—Final Report. NTIS Report No. PB89 178636/AS.

Strobel, S., and A. Ferguson. 1986. Induction of oral tolerance, in mice, to gum arabic. *Food Addit. Contam.* 3:43–46.

Strobel, S., A. Ferguson, and M. W. Anderson. 1982. Immunogenicity of foods and food additives—*In vivo* testing of gums arabic, karaya, and tragacanth. *Toxicol. Lett.* 14:247–252.

Strobel, S., A. Ferguson, and D. M. W. Anderson. 1986. Immunogenicity, immunological cross reactivity and non-specific irritant properties of the exudate gums, arabic, karaya and tragacanth. *Food Addit. Contam.* 3:47–56.

Tezuka, Y., K. Honda, A. H. Banskota, M. M. That, and S. Kadota. 2000. Kinmoonosides A-C, three new cytotoxic saponins from the fruits of *Acacia concinna*, a medicinal plant collected in Mayanmar. *J. Nat. Prod.* 63:1658–1664.

Tlolka-Pluszczyk, J. 1970. Induction of neoplasms in guinea pigs by intramediastinal administration of methylcholanthrene. *Pol. Med. J.* 10:1186–1200.

Trivedi, C. P., N. T. Modi, R. K. Sarin, and S. S. Rao. 1986. Bronchodilator and anti-inflammatory effect of glycosidal fraction of *Acacia farnesiana. Indian J. Physiol. Pharmacol.* 30:267–268.

United States Pharmacopeial Convention, Inc. 2000. National Formulary Specifications on Acacia. *National Formulary*, 2209. Rockville, MD: United States Pharmacopeial Convention, Inc.

van Ketel, W. G. 1984. Simultaneous sensitization to gum arabic and cobalt. *Contact Dermatitis* 10:180.

Vogel, H. H., and R. Zaldivar. 1971. Carcinogenesis: The interaction of chemical and physical agents. *Radiat. Res.* 47:644–659.

Waller, D. P., N. Bunyapraphatsara, A. Martin, C. J. Vournazos, M. S. Ahmed, D. D. Soejarto, G. A. Cordell, H. H. S. Fong, L. D. Russel, and J. P. Malone. 1983. Effect of (+)-gossypol on fertility in male hamsters. *J. Androl.* 4:276–279.

Wassel, G. M., S. M. Abd El-Wahab, E. A. Aboutabl, N. M. Ammar, and M. S. Afifi. 1992. Phytochemical examination and biological studies of *Acacia nilotica L. Wild and Acacia farnesiana L. Wild* growing in Egypt. *Egypt J. Pharm. Sci.* 33:327–340.

Wenninger, J. A., and G. N. McEwen, Jr., eds. 1995. *International Cosmetic Ingredient Dictionary*, 6th ed., Vol 1, 1–2. Washington, DC: CTFA.

Wenninger J. A, G. N. McEwen, Jr., eds. 1997. *International Cosmetic Ingredient Dictionary and Handbook*., 7th ed., Vol 1, 2–3. Washington, DC:CTFA.

West Coast Analytical Service, Inc. 1999. Laboratory report on Acacia gum (Lot AN-1292). Analysis: Selected pesticides by GC/ECD and GC/TSD. Unpublished data submitted by CTFA, July 31, 2000. (7 pages.)[2]

Wild, D., E. Gocke, D. Harnasch, G. Kaiser, and M-T. King. 1985. Differential mutagenic activity of IQ (2-amino-3-methylimidazo[4,5-f]quinoline) in *Salmonella typhimurium* strains *in vitro* and *in vivo*, in *Drosophila* and in mice. *Mutat. Res.* 156:93–102.

Winter, C. A., E. A. Riseley, and G. W. Nuss. 1962. Carrageenin-induced edema in hind paw of rat as an assay for anti-inflammatory drugs. *Proc. Soc. Exp. Biol. Med.* 3:544–547.

World Health Organization (WHO). 1974. *Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers, and thickening agents*, 316–319. Geneva: WHO.

World Health Organization (WHO). 1990. Gum arabic. *WHO Food Additives Series, 26. Toxicological evaluation of certain food additives and contaminants*, 35th ed., 77–79. Geneva: WHO.

Yegnanarayan, R., and G. V. Joglekar. 1978. Anti-fertility effect of non-steroidal anti-inflammatory drugs. *Japan J. Pharmacol.* 28:909–917.

Zeiger, E., B. Anderson, S. Haworth, T. Lawlor, and K. Mortelmans. 1992. *Salmonella* mutagenicity tests: V. Results from the testing of 311 chemicals. *Environ. Mol. Mutagen.* 19:2–141.

SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006

SECTION 1: Identification of the substance/mixture and of the company/undertaking

1.1 Product identifier

Trade name	:	Gum Arabic
Product code	:	A3553
EC-No.	:	232-519-5

1.2 Relevant identified uses of the substance or mixture and uses advised against

Use of the Substance/Mixture	:	Use as laboratory reagent

1.3 Details of the supplier of the safety data sheet

Company	:	TCI EUROPE N.V.
Address	:	Boereveldseweg 6 - Haven 1063, B-2070 Zwijndrecht, Belgium
Telephone	:	+32 (0)3 735 07 00
Telefax	:	+32 (0)3 735 07 01
E-mail address of person responsible for the SDS	:	sales-eu@tcichemicals.com

1.4 Emergency telephone number

Emergency telephone number	:	+44 844 892 0111

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

Classification (REGULATION (EC) No 1272/2008)

Not a hazardous substance or mixture.

2.2 Label elements

Labelling (REGULATION (EC) No 1272/2008)

Not a hazardous substance or mixture.

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.
Ecological information: The substance/mixture does not contain components considered to have endocrine disrupting properties according to REACH Article 57(f) or Commission Delegated regulation (EU) 2017/2100 or Commission Regulation (EU) 2018/605 at levels of 0.1% or higher.
Toxicological information: The substance/mixture does not contain components considered to have endocrine disrupting properties according to REACH Article 57(f) or Commission Delegated regulation (EU) 2017/2100 or Commission Regulation (EU) 2018/605 at levels of 0.1% or higher.

SECTION 3: Composition/information on ingredients

3.1 Substances

Substance name	:	Gum Arabic
EC-No.	:	232-519-5

Components

Remarks	:	No hazardous ingredients

SECTION 4: First aid measures

4.1 Description of first aid measures

If inhaled	:	Remove person to fresh air and keep comfortable for breathing. Get medical advice/ attention if you feel unwell.
In case of skin contact	:	Take off all contaminated clothing immediately. If on skin, rinse well with water. If skin irritation or rash occurs: Get medical advice/ attention.
In case of eye contact	:	Rinse with plenty of water. If easy to do, remove contact lens, if worn. If eye irritation persists: Get medical advice/ attention.
If swallowed	:	Get medical advice/ attention. Rinse mouth.

4.2 Most important symptoms and effects, both acute and delayed

None known.

4.3 Indication of any immediate medical attention and special treatment needed

None known.

SECTION 5: Firefighting measures

5.1 Extinguishing media

Suitable extinguishing media	:	Dry powder, Foam, Water spray, Carbon dioxide (CO2)

5.2 Special hazards arising from the substance or mixture

Specific hazards during fire-fighting	:	No information available.

5.3 Advice for firefighters

Special protective equipment for firefighters	:	Use personal protective equipment.
Specific extinguishing methods	:	Use extinguishing measures that are appropriate to local circumstances and the surrounding environment. Immediately evacuate personnel to safe areas. Remove undamaged containers from fire area if it is safe to do so.

SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures

Personal precautions	:	Wear suitable protective equipment. Keep people away from and upwind of spill/leak. Entry to non-involved personnel should be controlled around the leakage area by roping off, etc.

6.2 Environmental precautions

Environmental precautions	:	Prevent product from entering drains.

6.3 Methods and material for containment and cleaning up

Methods for cleaning up	:	Pick up and arrange disposal without creating dust.

6.4 Reference to other sections

See sections: 7, 8, 11, 12 and 13.

SECTION 7: Handling and storage

7.1 Precautions for safe handling

Technical measures	:	Prevent dispersion of dust.
Local/Total ventilation	:	Ensure adequate ventilation. Use a local exhaust ventilation.
Advice on safe handling	:	Avoid contact with skin, eyes and clothing. Wear personal protective equipment. Wash hands and face thoroughly after handling.

7.2 Conditions for safe storage, including any incompatibilities

Requirements for storage areas and containers	:	Keep container tightly closed. Store in a cool and shaded area. Protect from moisture. Keep under inert gas.

7.3 Specific end use(s)

Specific use(s)	:	No information available.

SECTION 8: Exposure controls/personal protection

8.1 Control parameters

Contains no substances with occupational exposure limit values.

8.2 Exposure controls

Engineering measures

Install a closed system or local exhaust.
Also install safety shower and eye bath.

Personal protective equipment

Eye/face protection	:	Safety glasses, Face-shield
Hand protection	:	Protective gloves
Skin and body protection	:	Protective suit
Respiratory protection	:	Dust mask

*Use personal protective equipment(PPE) approved under appropriate government standards and follow local and national regulations.

SECTION 9: Physical and chemical properties

9.1 Information on basic physical and chemical properties

Physical state	:	Solid form
Colour	:	white - yellow
Odour	:	No data available
Odour Threshold	:	No data available
Melting point/freezing point	:	No data available
Boiling point/boiling range	:	No data available
Flammability	:	No data available
Upper explosion limit/Upper flammability limit	:	No data available
Lower explosion limit/Lower flammability limit	:	No data available
Flash point	:	No data available
Auto-ignition point	:	No data available
Decomposition temperature	:	No data available
pH	:	No data available
Viscosity		
Viscosity, dynamic	:	No data available
Viscosity, kinematic	:	No data available
Solubility(ies)		
Water solubility	:	No data available
Solubility in other solvents	:	No data available
Partition coefficient: n-octanol/water	:	No data available
Vapour pressure	:	No data available
Relative density	:	No data available
Relative vapour density	:	No data available
Particle characteristics	:	No data available

9.2 Other information

No data available

SECTION 10: Stability and reactivity

10.1 Reactivity

No data available

10.2 Chemical stability

Stable under normal conditions.

10.3 Possibility of hazardous reactions

Hazardous reactions : None under normal processing.

10.4 Conditions to avoid

Conditions to avoid : Exposure to moisture,

10.5 Incompatible materials

10.6 Hazardous decomposition products

No data available,

SECTION 11: Toxicological information

11.1 Information on hazard classes as defined in Regulation (EC) No 1272/2008

Acute toxicity	:	No information available.
Skin corrosion/irritation	:	No information available.
Serious eye damage/eye irritation	:	No information available.
Respiratory or skin sensitisation	:	No information available.
Germ cell mutagenicity	:	No information available.
Carcinogenicity	:	No information available.
Reproductive toxicity	:	No information available.
STOT - single exposure	:	No information available.
STOT - repeated exposure	:	No information available.
Repeated dose toxicity	:	No information available.
Aspiration hazard	:	No information available.

11.2 Information on other hazards

Endocrine disrupting properties

Product:

Assessment : The substance/mixture does not contain components considered to have endocrine disrupting properties according to REACH Article 57(f) or Commission Delegated regulation (EU) 2017/2100 or Commission Regulation (EU) 2018/605 at levels of 0.1% or higher.

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

Product:

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

Product:

Assessment : The substance/mixture does not contain components considered to have endocrine disrupting properties according to REACH Article 57(f) or Commission Delegated regulation (EU) 2017/2100 or Commission Regulation (EU) 2018/605 at levels of 0.1% or higher.

12.7 Other adverse effects

No data available

SECTION 13: Disposal considerations

13.1 Waste treatment methods

Product : Disposal in accordance with local and national regulations.
Entrust disposal to a licensed waste disposal company.

Contaminated packaging : Disposal in accordance with local and national regulations.
Before disposal of used container, remove contents completely.

SECTION 14: Transport information

14.1 UN number or ID number

ADR : Not regulated as a dangerous good

IMDG : Not regulated as a dangerous good

IATA : Not regulated as a dangerous good

14.2 UN proper shipping name

ADR : Not regulated as a dangerous good

IMDG : Not regulated as a dangerous good

IATA : Not regulated as a dangerous good

14.3 Transport hazard class(es)

ADR : Not regulated as a dangerous good

IMDG : Not regulated as a dangerous good

IATA : Not regulated as a dangerous good

14.4 Packing group

ADR : Not regulated as a dangerous good

IMDG : Not regulated as a dangerous good

IATA (Cargo) : Not regulated as a dangerous good

IATA (Passenger) : Not regulated as a dangerous good

14.5 Environmental hazards

Not regulated as a dangerous good

14.6 Special precautions for user

Remarks : Not classified as dangerous in the meaning of transport regulations.

14.7 Maritime transport in bulk according to IMO instruments

Not applicable for product as supplied.

SECTION 15: Regulatory information

15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture

REACH - Restrictions on the manufacture, placing on the market and use of certain dangerous substances, mixtures and articles (Annex XVII)	:	Not applicable
Regulation (EC) No 649/2012 of the European Parliament and the Council concerning the export and import of dangerous chemicals	:	Not applicable
REACH - Candidate List of Substances of Very High Concern for Authorisation (Article 59).	:	Not applicable
Regulation (EC) No 1005/2009 on substances that deplete the ozone layer	:	Not applicable
Regulation (EU) 2019/1021 on persistent organic pollutants (recast)	:	Not applicable
REACH - List of substances subject to authorisation (Annex XIV)	:	Not applicable

Water hazard class (Germany) : WGK 2 obviously hazardous to water
Code Number: 8 055
Classification according to AwSV, Annex 1 (4)

The components of this product are reported in the following inventories:

CH BAGREG	:	On the inventory, or in compliance with the inventory
TSCA	:	All substances listed as active on the TSCA inventory
AIIC	:	On the inventory, or in compliance with the inventory
DSL	:	All components of this product are on the Canadian DSL
ENCS	:	Not in compliance with the inventory
ISHL	:	Not in compliance with the inventory
KECI	:	On the inventory, or in compliance with the inventory
PICCS	:	On the inventory, or in compliance with the inventory
IECSC	:	On the inventory, or in compliance with the inventory
NZIoC	:	On the inventory, or in compliance with the inventory

15.2 Chemical safety assessment

A Chemical Safety Assessment is not required for this substance.

SECTION 16: Other information

Full text of other abbreviations

ADN - European Agreement concerning the International Carriage of Dangerous Goods by Inland Waterways; ADR - Agreement concerning the International Carriage of Dangerous Goods by Road; AIIC - Australian Inventory of Industrial Chemicals; ASTM - American Society for the Testing of Materials; bw - Body weight; CLP - Classification Labelling Packaging Regulation; Regulation (EC) No 1272/2008; CMR - Carcinogen, Mutagen or Reproductive Toxicant; DIN - Standard of the German Institute for Standardisation; DSL - Domestic Substances List (Canada); ECHA - European Chemicals Agency; EC-Number - European Community number; ECx - Concentration associated with x% response; ELx - Loading rate associated with x% response; EmS - Emergency Schedule; ENCS - Existing and New Chemical Substances (Japan); ErCx - Concentration associated with x% growth rate response; GHS - Globally Harmonized System; GLP - Good Laboratory Practice; IARC - International Agency for Research on Cancer; IATA - International Air Transport Association; IBC - International Code for the Construction and Equipment of Ships carrying Dangerous Chemicals in Bulk; IC50 - Half maximal inhibitory concentration; ICAO - International Civil Aviation Organization; IECSC - Inventory of Existing Chemical Substances in China; IMDG - International Maritime Dangerous Goods; IMO - International Maritime Organization; ISHL - Industrial Safety and Health Law (Japan); ISO - International Organisation for Standardization; KECI - Korea Existing Chemicals Inventory; LC50 - Lethal Concentration to 50 % of a test population; LD50 - Lethal Dose to 50% of a test population (Median Lethal Dose); MARPOL - International Convention for the Prevention of Pollution from Ships; n.o.s. - Not Otherwise Specified; NO(A)EC - No Observed (Adverse) Effect Concentration; NO(A)EL - No Observed (Adverse) Effect Level; NOELR - No Observable Effect Loading Rate; NZIoC - New Zealand Inventory of Chemicals; OECD - Organization for Economic Cooperation and Development; OPPTS - Office of Chemical Safety and Pollution Prevention; PBT - Persistent, Bioaccumulative and Toxic substance; PICCS - Philippines Inventory of Chemicals and Chemical Substances; (Q)SAR - (Quantitative) Structure Activity Relationship; REACH - Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals; RID - Regulations concerning the International Carriage of Dangerous Goods by Rail; SADT - Self-Accelerating Decomposition Temperature; SDS - Safety Data Sheet; SVHC - Substance of Very High Concern; TCSI - Taiwan Chemical Substance Inventory; TECI - Thailand Existing Chemicals Inventory; TRGS - Technical Rule for Hazardous Substances; TSCA - Toxic Substances Control Act (United States); UN - United Nations; vPvB - Very Persistent and Very Bioaccumulative

Further information

This SDS was prepared sincerely based on the information obtained, however, any warranty shall not be given regarding the data contained and the assessment of hazards and toxicity. Prior to use, please investigate not only the hazards and toxicity information but also the laws and regulations of the organization, area and country where the products are to be used, which shall be given the first priority. The products are supposed to be used promptly after purchase in consideration of safety. Some new information or amendments may be added afterwards. If the products are to be used far behind the expected time of use or you have any questions, please feel free to contact us. The stated cautions are for normal handling only. In case of special handling operations, sufficient care should be taken, in addition to the safety measures suitable for the given situation. All chemical products should be treated with the recognition of "having unknown hazards and toxicity", which differ greatly depending on the conditions and handling when in use and/or the conditions and duration of storage. The products must be handled only by those who are familiar with specialized knowledge and have experience or under the guidance of those specialists throughout use from opening to storage and disposal. Safe usage conditions shall be set up on each user's own responsibility.

DE / 6N