FINAL REPORT

SAFETY ASSESSMENT OF ACACIA CATECHU, ACACIA CONCINNA, ACACIA CONCINNA EXTRACT, ACACIA DEALBATA, ACACIA DEALBATA EXTRACT, ACACIA DECURRENS, ACACIA DECURRENS EXTRACT, ACACIA FARNESIANA, ACACIA FARNESIANA EXTRACT, ACACIA SENEGAL, ACACIA SENEGAL EXTRACT, and ACACIA SENEGAL GUM EXTRACT

ABSTRACT

These ingredients are derived from various species of the acacia plant. The concentration at which these ingredients are reported to be used ranges from 9% in mascara to 1% in shampoos. Gum arabic is the common name for Acacia Catechu, Acacia Farnesiana, or Acacia Senegal, and describes material that exudes from the bark of the plant when it has been stressed by infection, poor nutrition, heat or drought. 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 addltive. Little information is available to characterize the extracts, however. Acacia Concinna Extract was generally described as containing saponins, alkaloids, and malic acid with parabens and potassium sorbate added as preservatives. The use of these ingredients categorized as biological additives, but no information was available to describe what function they serve in cosmetic formulations. 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. While there is extensive safety test data on gum arabic, it was not possible to relate these data to the crude Acacias and their extracts that are used in cosmetic formulations. Therefore, the available data were considered insufficient to support the safety of this family of ingredients in cosmetic products. The additional data need to complete the safety assessment 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. Additionally, determine 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) 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 studies may be needed after clarification of the chemical constituents of the Acacias.

INTRODUCTION

The safety for use in cosmetics of ingredients derived from species of Acacia and listed in the International Cosmetic Ingredient Dictionary and (Wenninger and McEwen, 1995a) is reviewed in this report. The ingredients and the Acacia species from which they are derived include: Acacia Catechu, Acacia Concinna, Acacia Concinna Extract, Acacia Dealbata, Acacia Dealbata Extract, Acacia Decurrens, Acacia Decurrens Extract, Acacia Farnesiana, Acacia Farnesiana Extract, Acacia Senegal, Acacia

and Acacia Senegal.

Gum Arabic is a substance that is generally recognized as safe (GRAS) for direct addition to human food under the provisions of Section 1884.1330 of the Code of Federal Regulations (21 CFR 1884.1330). A report, prepared for the Food and Drug Administration, 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 the following (FASEB, 1973): "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."

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** section for maximum values for possible daily human intake).

Studies from the 1973 FASEB report are summarized in the text of this report. Studies on Acacia Senegal and other species of Acacia (listed in International Cosmetic Ingredient Dictionary 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

Chemical And Physical Properties

The International Cosmetic Ingredient Dictionary (Wenninger and McEwen, 1997) is the source of the following descriptions of various species of Acacia:

Acacia Catechu - also known as Catechu and Gum Arabic, is the dried, crushed core of *Acacia catechu*. It is also defined as the plant material derived from *Acacia catechu*.

Acacia Concinna - defined as the plant material derived from *Acacia concinna*.

Acacia Concinna Extract - also Extract of Acacia Concinna, is an extract of the fruit of *Acacia concinna*.

Acacia Dealbata - defined as the plant material derived from *Acacia dealbata*.

Acacia Dealbata Extract - an extract of the leaves of the wattle, *Acacia dealbata*. This ingredient is also known as Extract of Acacia Dealbata, Extract of Wattle, and Wattle Extract.

Acacia Decurrens - defined as the plant material derived from *Acacia decurrens*.

Acacia Decurrens Extract - an extract of the acacia, *Acacia decurrens*.

Acacia Farnesiana - (CAS No. 9000-01-5), also known as Acacia and Gum Arabic, is a plant material that is derived from the dried, gummy exudate of the acacia, *Acacia farnesiana*. It is also defined as the plant material derived from *Acacia farnesiana*.

Acacia Farnesiana Extract - an extract of the flowers and stems of the acacia, *Acacia farnesiana*. This ingredient is also known as Extract of Acacia Farnesiana and Acacia Extract.

Acacia Senegal - (CAS No. 9000-01-5), also known as Acacia and Gum Arabic, is a plant material derived from the dried, gummy exudate of the acacia, *Acacia senegal*. It is also defined as the plant material derived from *Acacia senegal*.

Acacia Senegal Extract - also known as Acacia Extract and Extract of Acacia Senegal, is an extract of the flowers and stems of the acacia, *Acacia senegal*.

Acacia Senegal Gum Extract - an extract of the gum of the acacia, *Acacia senegal*. Synonyms for this ingredient include Acacia Gum Extract and Extract of Acacia Senegal Gum (Wenninger and McEwen, 1997).

In the preceding definitions from the International Cosmetic Ingredient Dictionary, Gum Arabic is another name for Acacia Catechu, Acacia Farnesiana, and Acacia Senegal. Information from another source defines Gum Arabic as the dried gummy exudate from the stems and branches of *Acacia senegal*, *Acacia arabica*, and other species of Acacia (Anonymous, 1993). The gummy exudate from Acacia Senegal has been described as a proteinaceous polysaccharide, with protein content ranging from approximately 1.5% to 3% for samples from various producing areas (World Health Organization, 1990).

Data on physical properties indicate that Gum Arabic is a white powder that is readily soluble in water, but insoluble in alcohol (Anonymous, 1993). It has a molecular weight of approximately 850,000 (Ross et al., 1984) and a density of 1.35 to 1.49 (Anonymous, 1981). The aqueous solution is acid to litmus (Lewis, 1993a). Other names for Gum Arabic include: Acacia, Acacia Gum, Acacia Dealbata Gum, Acacia Senegal, Acacia Syrup, Arabic Gum, Australian Gum, Gum Ovaline, Gum Senegal, Indian Gum, Senegal Gum, and Wattle Gum (Anonymous, 1981).

The structure of Gum Arabic has been defined as follows: "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→ 3) linkages and side chains or branched oligosaccharides linked to the main chain by β-D-(1→ 6) linkages. The oligosaccharides may contain terminal rhamnosyl units linked (1→ 3) or terminal arabinofuranosyl units linked (1→ 4) to internal galactosyl or glucuronosyl units." (Pazur et al., 1986)

Based on methylation and degradation studies of Gum Arabic (*Acacia senegal*) along with periodate oxidation and other confirmatory reactions, a structure (Figure 1) for this gum has been proposed (Informatics Inc., 1972).

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).

Gum Arabic in solid form is imported from the Sudan. According to one source, the solid is converted to a liquid form and 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 Extract) is drawn by cold processing (Carlisle International Corporation, 1997a).

COMPOSITION, ANALYTICAL METHODS, AND IMPURITIES

Acacia Senegal has been described as the major commercial Acacia gum (Anderson, 1988). 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).

The following specifications exist for United States Pharmacopoeia (USP) grade Acacia: loss on drying (15% max), total ash (4% max), arsenic (3 ppm), lead (0.001%), and heavy metals (0.004%) (United States Pharmacopeial Convention, Inc., 1995).

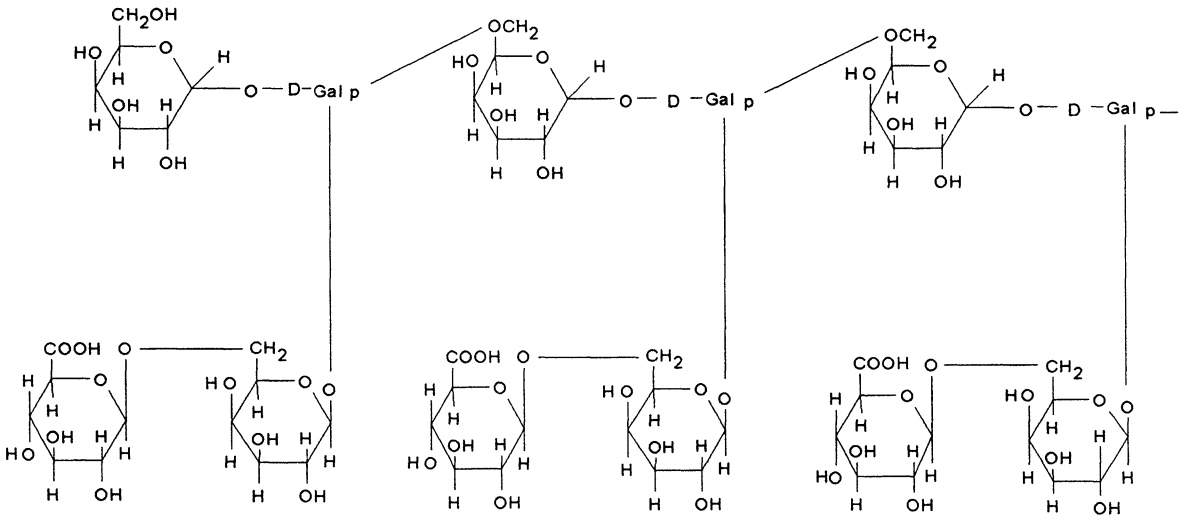

Figure 1. Proposed structure of Gum Arabic (Informatics, 1972).

FDA has listed Acacia (Gum Arabic) as a direct food addltive that meets the specifications of the "Food Chemicals Codex" (21 CFR 184.1330). The specifications for food grade Acacia include: arsenic (3 mg/kg max); ash, acid-insoluble (0.5% max); ash, total (4% max); heavy metals, as Pb (0.002% max); insoluble matter (1% max); lead (5 mg/kg max); and loss on drying (15% max) (Food Chemicals Codex, 1996). Data on the composition (impurities data included) of Acacia Senegal are included in Tables 1 through 4.

Information on the composition of various species of Acacia is included in Table 5. The Acacia species that are listed in the International Cosmetic Ingredient Dictionary are identified with an asterisk.

As noted in Table 5, aflatoxin has been detected in the bark and seeds of *Acacia catechu.* Furthermore, Gum-yielding *Acacia* twigs from the Sudan (supplier of Acacia Senegal) have been described as a source of aflatoxin (81 to > 1000 μg/kg) (Abdalla, 1988). However, the results of an enzyme-linked immunosorbent assay indicated no detectable aflatoxin in either of two samples of Gum Arabic. Absorbencies for both samples were equivalent to less than 2 ppb, the lowest detectable level. The assay system was capable of determining aflatoxin in the concentration range of 2.0 to 200.0 ppb in the presence of Gum Arabic (Smith et al., 1990).

The following information on Acacia Concinna Extract was received: Acacia Concinna Extract consists of 1 part of extract obtained from 1 part of dry pods of *Acacia concinna*. It contains the active

Table 1. Analytical Data for Natural Gum Arabic (*Acacia Senegal*) Samples provided by Importers in 1990/91 (Anderson et al.,1991)

Sample No.	Sample Sent by	Date received	% H_2O	% Ash	% N	Specific rotation[a] (degrees)	Conforms to revised JECFA Spec. (1990)[b]	Confirmation from NMR spectrum
N1	American importer A	12/90	13.2	3.8	0.34	-29	yes	Good *Acacia senegal*
N2	American importer A	12/90	13.9	4.0	0.36	-31	yes	Good *Acacia senegal*
N3	Italian importer B	12/90	14.4	3.3	0.31	-30	yes	Good *Acacia senegal*
N4	British importer C	12/90	14.5	3.6	0.37	-31	yes	Good *Acacia senegal*
N5	British importer D	12/90	12.2	3.5	0.35	-33	yes	Good *Acacia senegal*
N6	British importer E	12/90	14.9	2.0	0.38	-33	yes	Good *Acacia senegal*
N7	German importer A	1/91	14.4	4.0	0.26	-34	yes	Good *Acacia senegal*
N8	American importer G	1/91	15.0	3.2	0.29	-26	yes	Good *Acacia senegal*
N9	American importer H	1/91	13.9	3.9	0.33	-32	yes	Good *Acacia senegal*
N10	British importer K	2/91	13.3	3.7	0.34	-28	yes	Good *Acacia senegal*
N11	Italianimporter L	2/91	14.8	3.4	0.30	-29	yes	Good *Acacia senegal*
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).
[b]All samples conformed to the Revised (1990) JECFA (Joint Food and Agriculture Organization of the United Nations/World Health Organization Expert Committee on Food Additives) 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).

constituents of the pods of *Acacia concinna*, such as vegetable saponins. The raw material (*Acacia concinna*) from which Acacia Concinna Extract is derived is from wild, crafted sources. Thus, reportedly, there is no contamination of the raw material with pesticide residues (Carlisle International Corporation, 1997a). Specifications for Acacia Concinna Extract are listed in Table 6, and the analytical profile of this ingredient is included in Table 7.

REACTIVITY

When Gum Acacia was 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° destroys peroxidase (oxidizing agent) present in the gum and the colored derivatives produced (Gennaro, 1990).

USE

PURPOSE IN COSMETICS

The following species of Acacia function as biological additives in cosmetics: Acacia Catechu, Acacia Concinna Extract, Acacia Dealbata Extract, Acacia Decurrens Extract,

Table 2. Analytical Data For Sudanese and Nigerian Gum Arabic Samples of *Acacia senegal* (Anderson et al., 1990)

Properties/Composition	*Acacia senegal*	
	Sudanese samples Mean Values (13 years total between 1904 and 1989)	Nigerian samples Mean Values (9 years total between 1905 and 1967)
Equiv. weight - Corrected for moisture and protein contents	1050 ± 95	980 ± 56
Intrinsic viscosity (ml/g) - Corrected for moisture and protein contents	16 ± 2	18 ± 2
Brookfield viscosity, 25% (cp)	78 ± 13	84 ± 19
Specific rotation (degrees) - Corrected for moisture and protein contents	-30 ± 1.4	-30 ± 2
pH, 25% aqueous solution, at 25°C	4.4 ± 0.5	4.3 ± 0.03
Loss on drying, 105°C (%)	13 ± 0.8	13 ± 1
Total ash, 550°C (%)	3.6 ± 0.4	3.7 ± 0.3
Nitrogen (%)	0.34 ± 0.03	0.34 ± 0.03
Hence protein (%) - Corrected for loss on drying	2.3 ± 0.2	2.3 ± 0.4
Hence uronic anhydride (If all acidity arises from uronic acids)	17 ± 2	18 ± 1
Methoxyl (%) - Corrected for moisture and protein contents	0.25 ± 0.06	0.23 ± 0.03
4-O-Methylglucuronic Acid[a] (If all methoxyl content present in this acid)	1.5 ± 0.5	1.5 ± 0.3
Glucuronic Acid[a]	16 ± 1.7	16.6 ± 0.8
Galactose[a]	44 ± 6	47 ± 6
Arabinose[a]	25 ± 3	23 ± 4
Rhamnose[a]	14 ± 2	12 ± 2

[a]Sugar composition after hydrolysis (%) - Corrected for moisture and protein contents

Acacia Farnesiana, Acacia Farnesiana Extract, Acacia Senegal, Acacia Senegal Extract, and Acacia Senegal Gum Extract. The functions of Acacia Concinna, Acacia Dealbata, and Acacia Decurrens in cosmetics are not listed (Wenninger and McEwen, 1997).

Reportedly, *Acacia concinna* pods is a useful hair wash, in that it promotes hair growth, kills lice, and removes 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 Food and Drug Administration (FDA) in 1997

Table 3. The Amino Acid Composition of Sudanese and Nigerian Gum Arabic Samples of *Acacia senegal* (Anderson et al., 1990)

	Acacia senegal	
Amino Acid	Sudanese samples Mean Values[a] (13 years total between 1904 and 1989)	Nigerian samples Mean Values[a] (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

[a] (residues per 1000 residues)

indicated that Acacia was used in a total of 22 cosmetic products (Table 8) (FDA, 1997).

The following use concentration data on Acacia for various product categories were received from the cosmetics industry: Mascara (9%), Blush (1%), Make-up (1%), and Hair Mousse (1%) (CTFA, 1995).

Reportedly, recommended use concentrations of Acacia Concinna Extract are 0.5 to 5.0% w/w (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.

INTERNATIONAL USE

Acacia Senegal is listed in the *Japanese Comprehensive Licensing Standards of Cosmetics by Category* (*CLS*) (Rempe and Santucci, 1997). Acacia Senegal, which conforms to the specifications of the Japanese Cosmetic Ingredients Codex, has precedent for use without

Table 4. Cationic Composition of the Ash from Sudanese and Nigerian Gum Arabic Samples of *Acacia senegal* (Anderson et al., 1990)

	Acacia senegal	
Cation	Sudanese samples Mean Values[a] (15 years total between 1904 and 1989)	Nigerian samples Mean Values[a] (9 years total between 1905 and 1967)
Aluminum	190 ± 53	311 ± 156 (Mean = 266 [n = 8] if one value, 675, is treated as an outlier)
Calcium	256,000 ± 34,000	316,000 ± 56,000
Chromium	47 ± 22	34 ± 26
Copper	52 ± 27	66 ± 65 (Mean = 47, if one value, 225, is treated as an outlier)
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 (Mean = 25, if one value, 159, is treated as an outlier)
Arsenic	< 1 ppm	< 1 ppm
Cadmium	< 1 ppm	< 1 ppm
Cobalt	< 1 ppm	< 1 ppm
Molybdenum	< 1 ppm	< 1 ppm

[a] μg/g ash, unless expressed as ppm.

restriction in all CLS categories.

Acacia is not included among the substances listed as prohibited from use in cosmetic products that are marketed in the European Union (EEC, 1995).

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 1884.1330 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

Table 5. Composition Data on Various Species of Acacia

Acacia Species	Analytical Method	Components	Reference
*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). Flavonoids (all organs except stem). Cyanogenic glycosides (in pod, leaf, and stem). 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 nilotica (pod, leaf, stem, old stem, and flower)	Phytochemical screening	Carbohydrates and/or glycosides, reducing sugars, hydrolyzable tannins, condensed tannins, saponins, alkaloids and nitrogenous bases, unsaturated sterols, and/or terpenes, and coumarins (all organs). Volatiles (flower). Flavonoids (all organs except stem). Cyanogenic glycosides (pod and stem)	Wassel et al., 1992
Acacia latifolia (flowers)	Standard spectral, hydrolytic, and chromatographic data used	Flavonoids detected: quercetin 7-O-β-D-glucoside, quercetin 3-O-β-D-galactoside, quercetin 3-O-β-glucoside, quercetin 3-O-rutinoside, quercetin 3-O-trioside with galactose and glucose as sugars, myricetin 3-O-β-D-galactoside, myricetin 3-O-β-glucoside, taxifolin 7-O-α-D-glucoside, and isorhamnetin (after hydrolysis)	Voirin et al., 1986
Acacia leucophloea (stem bark)	- - -	n-hexacosanol, β-sitosterol, and β-amyrin	Khan et al., 1991
Acacia leucophloea (flower)	Column chromatography	Behenic ester, β-sitosterol, quercetin-3-glucoside, and mannitol	Khan et al., 1991
Acacia leucophloea (seed)	Column chromatography	Total free phenols (0.90 ± 0.03 g/100 g seed flour); tannins (0.68 ± 0.02 g/100 g seed flour)	Vijayakumari et al., 1994
Acacia leucophloea Wild. (leaves and pods)	Acid titration method	Hydrocyanic acid	Gupta and Nauriyal, 1966
*Acacia catechu** (bark)	Thin-layer chromatography and spectrophotometry	Aflatoxin B_1 (0.09 μg/g)	Roy and Kumari, 1991
*Acacia catechu** (seed)	Thin-layer chromatography and spectrophotometry	Aflatoxin B_1 (0.01 to 0.76 μg/g)	Roy and Kumari, 1991
Acacia berlandieri (leaf extract)	Paper chromatography	N-methyl beta-phenylethylamine (sympathomimetic)	Camp et al., 1963
Acacia berlandieri (leaves)	Paper, thin layer, and gas chromatography	Phenolic amines: tyramine, N-methyl-tyramine, and hordenine	Adams and Camp, 1966
Acacia berlandieri (leaves)	High performance liquid chromatography	Tyramine, N-methyltyramine, and hordenine	Pemberton et al., 1993
Acacia honey (species not stated)	Flame absorption spectrometry	Chromium (0.52 μg/g weight); ash (0.121%)	Petrovic et al., 1994

Acacia Species	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 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 georginae (seeds)	Extractive and chromatographic procedures	Fluoroacetic acid	Oelrichs and McEwan, 1962
Acacia atramen-taria and *Acacia tortuosa* (leaves)	Gas chromatography and NMR spectroscopy	Proacacipetalin (cyanogenic glucoside)	Seigler et al., 1983
Acacia aroma (leaves)	Gas chromatography and NMR spectroscopy	Linamarin and lotaustralin (cyanogenic glucosides)	Seigler et al., 1983
Acacia globulifera (leaves	Gas chromatography and NMR spectroscopy	Epiproacacipetalin (cyanogenic glucoside)	Seigler et al., 1983
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
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 species listed in International Cosmetic Ingredient Dictionary

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 thirty-fifth meeting of the Joint Food and Agriculture Organization of the United Nations/World Health Organization Expert Committee on Food Additives (JECFA), which was held in Rome from May 29 to June 7, 1989, JECFA confirmed its ADI (acceptable daily intake) "not specified" classification of Gum Arabic. Here, Gum Arabic (a.k.a. Gum Acacia) is defined as the dried gummy exudate from tropical and subtropical *Acacia senegal* trees.

The category ADI "not specified" is explained as follows: "This term 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

Table 6. Specifications for Acacia Concinna Extract (Carlisle International Corporation, 1997a)

Aspect	Brown, clear liquid
pH	4 to 6
Specific Gravity	1.01 to 1.1
Refractive Index (at 20°C)	1.1 to 1.4
Dried Residue (2 h /110°C)	5 to 10%
Water	60 to 65%
Propylene Glycol	35 to 40%
Water Solubility	Soluble
Preservatives	Parabens and Potassium Sorbate
Heavy Metal	< 10 ppm
Other Constituents	Saponins (detected by HPTLC)
UV/VIS Spectrophotometry (Absorbance at 220 nm of a 0.20% aqueous solution)	2.0 ± 0.20
Maximum Total Bacterial Count	100/g
Maximum Yeasts and Moulds	0/g

Table 7. Analytical Profile of Acacia Concinna Extract (Carlisle International Corporation, 1997a)

SPECIFICATION	STANDARD	SAMPLE
Aspect	Brown	Brown
pH	4 to 6	Passes
Specific Gravity (at 25°C)	1.0 to 1.10	Passes
Refractive Index (at 20°C)	1.1 to 1.4	Passes
Dried Residue (2 h/110°C)	10 to 20%	Passes
Water	60 to 65%	Passes
Propylene Glycol (%)	35 to 40%	Passes
Water Solubility	Soluble	Passes
Preservatives	Present	Passes
Heavy Metal	< 10 ppm	Passes
UV/VIS Spectrophotometry (Absorbance at 220 nm of a 0.1% aqueous solution)	1.0 ± 0.25	Conforms
HPTLC Method	Saponins, alkaloids, malic acid	Conforms
Maximum Total Bacterial Count	100/g	Passes
Maximum Yeasts and Moulds	0/g	Passes

Table 8. Product Formulation Data on Acacia (FDA, 1997)

Product Category	Total No. of Formulations in Category	Total No. Containing Ingredient
Bath Oils, Tablets, and Salts	141	1
Mascara	158	12
Other Eye Make-up Preparations	116	2
Other Hair Coloring Preparations	56	1
Foundations	283	1
Lipstick	758	1
Body and Hand Skin Care Preparations (Excl. Shaving)	776	2
Moisturizing Skin Care Preparations	743	1
Paste Masks (Mud Packs)	247	1
1997 Totals		22

health. For that reason, and for reasons stated in individual evaluations, the establishment of an acceptable daily intake expressed in numerical form is not deemed necessary. An addltive 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." (World Health Organization, 1990)

Gum Arabic (Acacia Senegal) 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). Furthermore, 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 chillies 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 pods reportedly is used in the treatment of skin diseases (Carlisle International Corporation, 1997b).

BIOLOGICAL PROPERTIES

ABSORPTION, DISTRIBUTION, AND METABOLISM

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 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).

The metabolism of Gum Arabic was evaluated 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 lib*, respectively, for four 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 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., 1984).

While the preceding study suggested that Gum Arabic was metabolized by bacteria in the cecum, the fate of undigested gum was not determined in earlier feeding studies involving rats and guinea pigs (Informatics, 1972).

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 ten 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. Non-lethal 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., 1984) 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. [See section under Clinical Assessment of Safety for human studies on absorption, distribution and excretion.]

HYPOTENSIVE ACTIVITY

The hypotensive activity of *Acacia catechu* (aqueous extract of branches) was evaluated using four groups of four anesthetized dogs (males and females; weights = 8 to 12 kg). The right femoral artery was cannulated for blood pressure recordings and, the right femoral vein, for 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 (Sham et al., 1984).

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).

In an *in vitro* experiment, *Acacia catechu* induced a dose-dependent relaxation of helical strips of rat tail artery that had been pre-constricted 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

The hypocholesterolemic activity of the dried water extract of *Acacia catechu*, also known as katha in India, was evaluated using three groups of ten 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 was killed after six weeks of feeding, and the remaining animals were killed after twelve 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 six months. In animals fed stock diet supplemented with cholesterol and katha for six months, the elevation of serum and liver cholesterol levels was significantly lower ($p = 0.001$) when compared to rats fed stock diet supplemented with cholesterol.

However, at the end of twelve 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

The hypoglycemic activity of ethanolic extracts of the pod, leaf, stem, old stem, and flower of *Acacia farnesiana L. Wild* was evaluated 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

The effect of ethanolic extracts of the pod, leaf, stem, old stem, and flower of *Acacia farnesiana L. Wiild* on uterine motility was evaluated. 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 organ extracts (in polysorbate 80) were added to organ water baths, respectively. Organ extracts were administered 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 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).

The bronchodilator activity of *Acacia farnesiana* was evaluated 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 (Trivedi et al., 1986).

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 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

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 vitro* (% inhibition of 38.2 and 26.26, respectively; $P < 0.001$, n = 10). It was concluded that this fraction has a promising anti-inflammatory

effect (Trivedi et al., 1986).

BIOCHEMICAL EFFECTS

Gum Arabic was administered twice daily to groups of four rats (weights = 100 to 110 g) at concentrations of 1, 2, and 10%, respectively, five days per week for four 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 x 20, 2 x 40, and 2 x 200 mg/kg/day, respectively. 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 x 200 mg/kg/day) marked uncoupling of oxidative phosphorylation was observed in the heart and liver after two 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 the study results in the preceding paragraph and decided to investigate whether comparable biochemical effects of Gum Arabic 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).

Trypsin inhibitor has been isolated from the seeds of *Acacia confusa* (Lin and Lin, 1985; Lin et al., 1991).

ANTIMICROBIAL ACTIVITY

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 fungi 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).

TOXICOLOGY

ACUTE ORAL TOXICITY

In an acute oral toxicity study using rabbits (weights and strain not stated), an Acacia Gum LD50 of 80 g/kg was reported (Dangerous Properties of Industrial Materials Report, 1981).

ACUTE INTRAPERITONEAL TOXICITY

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

The oral toxicity of Gum Arabic was evaluated using three-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 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).

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).

In another study, 10% (w/w) Gum Arabic (Acacia Senegal) was fed to Wistar albino rats (no. not stated; weights = 99 to 120 g) daily for 45 days. 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).

Three groups of three male Albino Wistar rats (weights = 140 to 160 g) were fed diets containing 1%, 4%, and 8% (w/w) Gum Arabic (Acacia Senegal), 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 investi-gators 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).

Diets containing Gum Arabic were fed to 133 guinea pigs. Twenty-two of the diets contained 15% Gum Arabic and one contained 20% Gum Arabic. The animals were fed for periods ranging from three to nine weeks. No toxic effects resulted from the administration of Gum Arabic (Informatics Inc., 1972).

SHORT-TERM INTRAVENOUS TOXICITY

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 highest dose; death occurred four months after the last injection. The cause of death was not determined. 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

The subchronic oral toxicity of Gum Arabic (Acacia Senegal) was evaluated 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 fifteen 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 females 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 combined 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 ($P < 0.01$). 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 ($P < 0.05$). At the highest dietary doses tested (~ 18%, 14 g/kg/day), kidney weights for male and female rats were significantly reduced ($P < 0.01$). 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, four groups of five male Albino Wistar rats (weights = 40 to 60 g) were fed diets containing 0.5, 1.5, 2.5, and 3.5% (w/w) Gum Arabic (Acacia Senegal), 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 (2 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).

IMMUNOLOGICAL RESPONSES

Studies on immunological responses to Gum Arabic are summarized in Table 9.

The allergenicity of Acacia solution (exact composition not stated) was evaluated using six rabbits (12 weeks old). The rabbits 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 three weeks later, and then three weeks after this injection at a dose of 15 cc. No signs of anaphylaxis were observed in this animal (Maytum and Magath, 1932).

In a second experiment (same study) evaluating the allergenicity of Acacia solution (exact composition not stated), eight guinea pigs (weights = 300 g) were injected intraperitoneally with a dose of 10 cc, and this dose was repeated 5, 12,

Table 9. Immunological Responses

Test Substance	Animals Tested	Test Procedure	Test Results	References
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 two surviving animals injected intracardially (0.5 cc); one died. Mild signs also in two of remaining four 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 four and three animals, respectively; extremely severe signs in two animals; three 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).	Three animals died at 2 to 3 min post-injection. The remaining two 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 seven-week period. After two 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 four-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
7% Gum Acacia solution	Two groups of 10 guinea pigs	Group 1: Injected subcutaneously (5 ml) repeatedly over 16-day period. Actively sensitized after seven doses and challenged in three weeks. Group 2: Received 11 subcutaneous injections. Passively sensitized and challenged 48 h later	Group 1: One animal with signs of asphyxia; Eight animals with shock signs; two 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 one month later, died of anaphylactic shock	Silvette et al., 1955

Test Substance	Animals Tested	Test Procedure	Test Results	References
Three grades of Gum Arabic (dissolved in 0.15 M NaCl at concentration of 4 mg/ml)	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] x 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 Freund's complete adjuvant (FCA) 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] x DBA/2 F_1] (BDF_1) mice	Footpad swelling test. Non-immunized 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 non-specific irritant effect	Strobel et al., 1986
Five different samples of Gum Arabic (*Acacia senegal*), each emulsified in FCA	5 groups of 30 to 40 [(C57BL/6J] x 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 FCA	5 groups of 30 to 40 [(C57BL/6J] x 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

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 sec. The two animals died approximately three minutes 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 (Maytum and Magath, 1932).

A follow-up experiment to the preceding study was performed to determine whether the deaths reported were due to the intravenous method of test substance administration in the first experiment (rabbits). 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, the four remaining guinea pigs that were injected intraperitoneally, the four guinea pigs that were injected intravenously in the first experiment, and four new guinea pigs were injected intraperitoneally with Acacia solution (10 cc). Of the four new guinea pigs, two died from peritonitis within four days.

Seven days after intraperitoneal injection, the remaining ten animals from the third experiment were injected intraperitoneally with 10 cc Acacia. Four of the ten died of peritonitis on the next day. Therefore, Acacia was capable of inducing peritonitis (followed by death) only after intraperitoneal administration (Maytum and Magath, 1932).

The results of studies involving a total of 19 guinea pigs (8 guinea pigs from preceding experiment included) include sensitization induced by Acacia solution (administered parenterally; exact composition not stated) in a total of 19 guinea pigs, 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 three weeks should be given cautiously because of the possibility of anaphylactic reactions (Maytum and Magath, 1932).

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).

The effect of Gum Acacia on complement and antibody production was evaluated using two groups of ten 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 two 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 six weeks, bled again, injected with a fifth dose of vaccine, and bled for a fourth time seven 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 two 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, both 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).

The effect of Gum Acacia on complement and antibody production was also evaluated using four rabbits (weight range = 1800 to 2650 g). This experiment is from the study in the preceding paragraph. The rabbits were injected subcutaneously with a 7% solution of Gum Acacia (10 ml) every second day for four weeks. All rabbits were bled on the fifteenth day and injected

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 non-immunized 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 agglutinative and complement-fixing activity in the sera to the bacterial antigen *Brucella abortus* (Rice, 1954a).

In another study, complement titers were evaluated 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 solution over a period of 16 days. The animals were actively sensitized after 7 doses, and the nine survivors were bled, challenged, and re-bled in three 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 very excited shortly after challenge, running around wildly and squealing (shock signs); two eventually died. An additional ten guinea pigs that had received eleven 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).

Twelve guinea pigs (strain not stated; weights ≈ 300 g) were sensitized via single intra-abdominal injections of 600 mg Acacia (6% solution, 10 m; exact composition of solution not stated). The animals were challenged one month later with an intravenous injection of 60 mg of the sensitizing sample or other samples of Acacia.

Anaphylactic shock resulted in each of the twelve guinea pigs, ten of which died. Two additional guinea pigs were sensitized via intra-abdominal injection of 160 mg Acacia with Freund's complete adjuvant (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 one 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 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. 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).

Additionally, Narita (1985) reported the isolation of high-titer anti-Gum Arabic sera were obtained from rabbits injected with Gum Arabic. The antisera had cross-reactivity with the Lewis^a antigen (Le^a) antigen, as measured by both a single diffusion tube test and the Ouchterlony test (Narita, 1985).

The allergenicity of three grades of Gum Arabic

was evaluated using female CBA mice (6 weeks old; 6 to 8 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 mice (4 mice per antigen) did not induce significant footpad swelling at 24 h. Similarly, the intradermal injection of saline into immunized mice did not cause a significant increase in footpad thickness. However, compared to the control, significant positive responses were noted ($P < 0.01$), 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 ($P < 0.005$) when compared to the colorless grade (Strobel et al., 1982).

The immunological activity of Gum Arabic was evaluated using two groups of eight female BDF1 [(C57BL/6] x 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 seven 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 three weeks after immunization all mice were bled and decomplemented sera were tested for anti-Gum Arabic antibodies by a micro-ELISA technique (Strobel et al., 1982). Delayed-type hypersensitivity was also measured (skin test) at three 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 pre-fed) mice after systemic immunization. However, antibodies were not detected in the serum of mice that were pre-fed with Gum Arabic. Regarding delayed type hypersensitivity, a similar pattern was noted. Positive skin tests were reported for all saline-pre-fed mice. However, footpad swelling in mice pre-fed with Gum Arabic was significantly suppressed. Test results indicated that systemic immunological hypo-responsiveness (oral tolerance) developed in mice that were fed Gum Arabic (Strobel and Ferguson, 1986).

The immunogenicity, cross-reactivity, and non-specific irritant properties of Gum Arabic (*Acacia senegal*) were evaluated using male mice (6 to 8 weeks old) of the [(C57BL/6J x DBA/2F_1] (BDF_1) strain. Non-specific irritant properties were assessed in the foot pad swelling test using control groups of non-immunized 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 non-specific footpad swelling test, five groups (6 to 8 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, non-specific swelling ($P < 0.05$). Sample E induced the greatest extent of footpad swelling. These results (footpad swelling) were indicative of a non-specific irritant effect (Strobel et al., 1986).

In the 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 Freund's complete adjuvant prior to immunization. Control mice (30 to 40 mice) were immunized with saline in Freund's complete adjuvant. 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) three weeks after immunization. Antibodies were assayed by an enzyme-linked immunosorbent assay (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 non-specific swelling was produced by Samples B and C (Strobel et al., 1986).

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 (2 animals, 7 months old). These 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 γ-globulin. At microscopic examination, a severe inflammatory response was observed in both germ-free and conventional 8-day-old guinea pigs. 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 γ-globulin concentrations. The extent of the inflammation induced by Acacia paralleled γ-globulin concentrations in germ-free guinea pigs more closely than in conventional guinea pigs (Aronson and McMaster, 1972).

MUTAGENICITY

Both *in vitro* and *in vivo* studies on the mutagenicity of Gum Arabic are summarized in Table 10. While a few positive results are described, most studies were negative for mutagenic activity.

ANTIMUTAGENICITY

The antimutagenic activity of *Acacia arabica* was evaluated using the WP-2 strain of *Escherichia coli.* UV light was used as the mutagen. Cultures were irradiated with UV light (1.5 J/m^2/ sec) for 15 sec, 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).

ENHANCEMENT OF MUTAGENICITY

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

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 four to ten 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).

The carcinogenicity of Gum Arabic was evaluated using four-week-old F344 rats (50 males, 50 females) and four to five-week-old B6C3F$_1$ mice (50 males, 50 females) in a two-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 one-to-two 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. Study results are summarized below (Melnick et al., 1983):

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 (Melnick et al., 1983).

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

Table 10. *In vitro* and *in vivo* mutagenicity of Gum Arabic and *Acacia belandieri*.

Test Substance	Bacterial strains/cells Tested	Test Procedure	Test Results	References
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
Gum Arabic	*Salmonella typhimurium* G-46 and TA-1530	Ames test (Ames, 1971)	Not mutagenic	Maxwell and Newell, 1973
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 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* 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, 1973
Gum Arabic	*Saccharomyces cerevisiae* D3	Plate test (Brusick, 1973)	Not mutagenic	Green, 1977
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	Slightly positive. Further tests and detailed statistical evaluation needed to confirm classification	Maxwell and Newell, 1973
Gum Arabic	WI-38 human embryonic lung cells	Test methodology not stated	Chromosomal aberrations induced in anaphase	Green, 1977
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	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. Biological significance of these data could be questioned	Sheu et al., 1986
Gum Arabic	(SEC X C57BL)F1 and (C3H X C57BL)F1 female mice (10 to 12 weeks old. (101 X C3H)F1 male mice (8 weeks old)	Dominant lethal test. Male rats fed diets containing up to 20% Gum Arabic prior to mating	No evidence of dominant lethal effect	Sheu et al., 1986
Gum Arabic	Male and female Swiss mice (10 to 12 weeks old; weights = 25 to 30 g)	Dominant lethal test. Male rats dosed orally with 1% Gum Arabic prior to mating	No dominant lethal effect	Kar et al., 1984

Test Substance	Bacterial strains/cells Tested	Test Procedure	Test Results	References
Gum Arabic	(SEC X C57BL)F1 female mice (10 to 12 weeks old. (101 X 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	Sheu et al., 1986
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	Results slightly positive in acute and short-term assays. Further tests and detailed statistical evaluation needed to confirm this possibility	Maxwell and Newell, 1973
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
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
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
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
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 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
Gum Arabic	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	Wild et al., 1985
Gum Arabic	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	Wild et al., 1985

(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 ($P < 0.05$) in tumor incidence was observed in the high dose group, and this was the only statistically significant finding for this neoplasm (Melnick et al., 1983).

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 (Melnick et al., 1983).

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

The cocarcinogenicity of Gum Acacia was evaluated 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 ± 39.4 days, $n = 30$) was significantly shortened when compared to untreated controls (669.2 ± 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 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 six 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

Studies on the reproductive and developmental toxicity of Gum Arabic are summarized in Table 11.

The antifertility activity of Gum Acacia (1 ml in water) was evaluated using ten female rats (strain and weights not stated). The test substance was administered by stomach tube daily for a period of five days after mating. After performing laparotomy on anesthetized dams, the number of fetuses was counted on the tenth 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).

The reproductive toxicity of Gum Acacia was evaluated using two groups of five male albino

Table 11. Reproductive and Developmental Toxicity Studies

Test Substance	Animals/cells Tested	Test Procedure	Test Results	References
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
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 Re-search Laboratories, 1972
Gum Arabic	Groups of female rats, rabbits, and hamsters	Oral doses of 16, 75, 350, and 1600 mg/kg on days 6 through 10 of gestation (hamsters and 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 the number occurring spontaneously in sham-treated controls	Food and Drug Re-search Laboratories, 1972
Gum Arabic (Acacia Senegal)	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
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 six days.	Normal microscopic variations in blastocysts reported: minor trophoblastic vacuolation, trophoblastic degeneration granules, and trophoblastic knob formations	Schardein et al., 1965
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
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
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 between experimental and control groups. No anti-fertility effect in males	Yegnanarayan and Joglekar, 1978

Test Substance	Animals/cells Tested	Test Procedure	Test Results	References
4% Gum Acacia	Adult female rabbits (weights between 1 to 2 g)	Dosed orally with 4% Gum Acacia for two days	No inhibitory effect on ovulation	Yegnanarayan and Joglekar, 1978
4% Gum Acacia	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 pro-estrus 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 pro-estrus stage of seventh 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	Yegnanarayan and Joglekar, 1978
4% Gum Acacia	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	Yegnanarayan and Joglekar, 1978
4% Gum Arabic	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	Yegnanarayan and Joglekar, 1978
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
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

rats of the Wistar strain (4 months old; weights between 180 to 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 following tissues were excised, homogenized, and centrifuged: testis, epididymis (divided into caput and cauda), seminal vesicle, ventral prostate, and coagulating gland. 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. Increased glycogen and LDH in the testis are both consequences of spermatogenic arrest. Decreased ACPase and increased ALPase activities in the testis also reflect the suppression of spermatogenesis. Gum Acacia did not induce suppression of spermatogenesis in this study (Akbarsha and Manivannan, 1993).

The teratogenicity of Gum Arabic was evaluated using six 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 Caesarean 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 10X 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 (Food and Drug Research Laboratories, 1972).

In the above 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 on gestation days 6 through 10. C-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) on days 6 through 18 of gestation; C-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). Positive control hamsters and rats received 250 mg aspirin.

The conclusion in the preceding paragraph was found to be applicable to all test groups of rats and hamsters. However it was concluded that 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. Maternal toxicity was noted at doses of 173 and 800 mg/kg; for surviving rabbits at these doses, the offspring were normal in all respects (Food and Drug Research Laboratories, 1972).

The teratogenicity of Gum Arabic (Acacia Senegal) was evaluated using groups of four-week-old Osborne-Mendel (FDA strain) rats. Beginning at thirteen 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, and included one female (7.5% dietary group) with a cystic ovary and one with lung nodules (15% dietary group). Sporadic non-significant increases in body weight were observed in all experimental groups (Collins et al., 1987). Additional results, summarized below, indicate that Gum Arabic was not a reproductive or developmental toxicant in rats at dietary concentrations of 1, 2, 4, 7.5 or 15%:

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 (Collins et al., 1987).

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 (Collins et al., 1987).

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 (Collins et al., 1987).

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 (Collins et al., 1987).

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 (Collins et al., 1987).

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).

A 10% aqueous *Acacia* solution was administered 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 six 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 (8 of 9 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, and included minor trophoblastic vacuolation, trophoblastic degeneration granules, and trophoblastic knob formations (Schardein et al., 1965).

The teratogenicity of a 5% solution of Gum Arabic (powder) in distilled water was evaluated 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).

The effect of a 5% solution of Gum Arabic (powder) in distilled water on fertility and general reproductive performance was evaluated 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 assigned to the natural delivery phase to raise

their young 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 three days after the last dose. As determined by analysis of testis sections, spermatogenesis was reported for all mice. All of the mice produced morphologically normal sperm, which were also observed in the epididymis (Waller et al., 1983).

Anti-fertility effects of 4% Gum Acacia were evaluated in a series of five experiments using male and female rats and female rabbits of the Haffkine strain. Six male albino rats (weights = 175 to 225 g) were tested In the first experiment. The rats were dosed orally daily for 28 days using a rubber catheter. Beginning on the first day of feeding, males were mated (1 male to 2 females) with females for twelve 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, and allowed to deliver normally.

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. However, this difference was not statistically significant (Yegnanarayan and Joglekar, 1978).

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 ten female rats over a period of two estrus cycles, beginning on the day of proestrus. The females were mated singly with males during the pro-estrus 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 pro-estrus 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 (Yegnanarayan and Joglekar, 1978).

The third experiment, for determining anti-implantation effects, involved 10 fertile rats (weight range from 150 to 200 g) that were mated in pro-estrus 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 (Yegnanarayan and Joglekar, 1978).

The fourth experiment was performed to determine any post-implantation effect of 4% Gum Arabic using ten fertile rats (weights = 150 to 200 g). After mating, the uteri were surgically exposed on the tenth day of pregnancy and the number of implantation sites counted. Female rats were dosed orally with 4% Gum Arabic on days 10 to 16 and litter sizes 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 ten 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 (Yegnanarayan and Joglekar, 1978).

In the fifth experiment, the anti-ovulatory 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 two 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 pre-treated 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, 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 5 injections), subcutaneously (5 injections), or administered orally (5 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 Caesarean 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

No evidence of the absorption of intact Gum Arabic was 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 (FASEB, 1973).

In a nephrotic patient, 20% of the Gum Arabic injected intravenously over a period of six weeks was excreted in the urine (FASEB, 1973).

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 non-lethal effects, were noted (FASEB, 1973).

The excretion of Gum Arabic and its effect on glucose absorption and routine hematological and biochemical measurements was evaluated using 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 one day of the control period and on one day during the third 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 feces 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 three weeks of administration. Additional study results are summarized in the following paragraph (Ross et al., 1983).

No significant differences in the mean concentration of serum lipids (phospholipids and triglycerides) were noted when concentrations before and after Gum Arabic ingestion were compared. However, a significant decrease in serum cholesterol (0.39 mmol/l reduction; $p < 0.05$) 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 vs. after Gum Arabic ingestion). Alanine amino transferase and aspartate amino transferase activities were significantly reduced ($p < 0.0025$; $p < 0.001$) 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., 1983).

SHORT-TERM ORAL TOXICITY

Five healthy male subjects (30 to 55 years old) ingested 25 g Gum Arabic (Acacia Senegal) 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 was administered to nine patients with nephrotic edema over periods up to eight 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 ten to 30 days, respectively (World Health Organization, 1974).

CLINICAL IMMUNOLOGY

Allergic disorders were reported for ten 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 ten subjects. The results of serologic studies (sera from 4 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).

Prick test results indicated that 24% of 228 subjects (ages not stated) with rhinitis and/or asthma who were patients at an allergy clinic in Australia had positive reactions to *Acacia longifolia*. A mean wheal size of 5.8 mm was reported. Two hundred of the patients had been studied for three years, whereas, the remaining 28 had been studied for six weeks (Kijvanit and Walls, 1986).

The skin sensitization potential of Acacia pollen extract (1000 protein nitrogen units/ml) was evaluated using a total of 36 patients in Brazil with asthma and/or rhinitis. The mean ages of the two test populations, from different parts of the country, were 25.7 years (20 patients) and 18.4 years (16 patients). All patients were tested intradermally (procedure not stated).

Of the 20 patients tested, three had mild intradermal skin test reactions to Acacia pollen extract. One of 16 patients in the remaining test population had a mild intradermal skin test reaction to Acacia pollen extract (Geller and Rosario, 1981).

Cross-reactivity between Gum Acacia and gum tragacanth was reported 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 IgE to pulverized Quillaja bark, Gum Arabic, and gum tragacanth were measured according to a modification of the radioallergosorbent test (RAST) (Wide et al., 1967). 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) who had never been 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).

CASE REPORTS

Case reports on Gum Arabic and other species of *Acacia* are summarized in Table 12. While two fatalities are reported, most case reports involve sensitization reactions.

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 2h 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	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

Ingredient Studied	Patients Evaluated	Procedure/Route of Exposure	Results	Reference
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 litho-printer 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

SUMMARY

This safety assessment is on the following species of Acacia that are listed in the International Cosmetic Ingredient Dictionary: Acacia Catechu, Acacia Concinna, Acacia Concinna Extract, Acacia Dealbata, Acacia Dealbata Extract, Acacia Decurrens, Acacia Decurrens Extract, Acacia Farnesiana, Acacia Farnesiana Extract, Acacia Senegal, Acacia Senegal Extract, and Acacia Senegal Gum Extract. According to the International Cosmetic Ingredient Dictionary, Gum Arabic is another name for Acacia Catechu, Acacia Farnesiana, and Acacia Senegal. Gum Arabic is generally recognized as safe (GRAS) for direct addition to food for human ingestion. Acacia Senegal has been described as the major commercial Acacia gum.

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→ 3) linkages and side chains or branched oligosaccharides linked to the main chain by β-D-(1→ 6) linkages. It 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.

Aflatoxin has been detected in the bark and seeds of Acacia Catechu (a.k.a. Gum Arabic). However, the results of an enzyme-linked immunosorbent assay indicated no detectable aflatoxin in either of two Gum Arabic samples analyzed. The limits of detection for aflatoxin in this assay system were in the concentration range of 2.0 to 200.0 ppb.

Information on Acacia Concinna Extract represents the only data on the Acacia ingredient family that were received from the cosmetics industry. These data indicate that Acacia Concinna Extract consists of 1 part of extract obtained from 1 part of dry pods of *Acacia concinna*. Active constituents in the pods include saponins, alkaloids, tannins, and malic acid. The raw material (*Acacia concinna*) from which Acacia Concinna Extract is derived is from wild, crafted sources. Thus, reportedly, there is no contamination of the raw material with pesticide residues.

According to an industry specification and analytical data sheet, Acacia Concinna Extract contains preservatives (e.g. parabens and potassium sorbate), saponins, alkaloids, and malic acid.

All of the species of Acacia reviewed in this report function as biological additives in cosmetics. Product formulation data submitted to the Food and Drug Administration in 1997 indicated that Acacia was used in 22 cosmetic products. Use concentrations of Acacia supplied by the cosmetics industry in 1995 included: Mascara (9%), Blush (1%), Make-up (1%), and Hair Mousse (1%).

Reportedly, *Acacia concinna* pods is a useful hair wash. The active constituents of *Acacia concinna* pods (saponins, alkaloids, tannins, and malic acid) are said to have cleansing 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).

Recommended use concentrations of Acacia Concinna Extract, derived from *Acacia concinna* pods, that have been reported include: 1.0 to 2.0% (for use in shampoos, hair packs, hair conditioners, and hair rinses) and 0.5 to 5.0% w/w.

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. Non-lethal effects included disturbances in hemoglobin values, white blood cells, and serum proteins.

Based on absorption and metabolism studies from a report, prepared for the Food and Drug Administration, affirming the GRAS status of Gum Arabic as a direct food addltive, it was 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. Studies in the preceding two paragraphs were also used in this assessment.

Dose-dependent uncoupling of oxidative phosphorylation was noted in groups of rats dosed orally with Gum Arabic at concentrations of 1, 2, and 10% twice daily for four weeks. Effects on oxidative phosphorylation were determined *in vitro* using cardiac and hepatic mitochondria. Damage to cardiac mitochondria progressed as dosing continued; however, hepatic mitochondrial function seemed to have gradually returned to normal during the fourth week of dosing. It was concluded that comparable biochemical effects were not observed *in vivo*, based on negative results for *in vivo* demethylation of 4-dimethyl(^{14}C)-aminoantipyrine.

An acute oral LD50 of 8000 mg/kg was reported for Acacia Gum in rabbits.

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 nine 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 non-toxic, 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, the only treatment-related alteration noted in rats at necropsy was cecal enlargement in animals of the highest dose groups. The highest dose groups consisted of male rats fed an average dietary concen-tration of 18.6% (dose = 14 g/kg/day) and female rats fed an average dietary concentration of 18.1% (dose = 13.8 g/kg/day). Kidney weights were significantly reduced in male rats fed 8.6% Acacia Senegal (5.22 g/kg/day) and in male and female rats of the highest dose groups. The reduction in liver weight noted in male rats was not significant. No significant hematological changes were observed between test and control groups. At microscopic examination, no alterations attributable to the ingestion of Acacia Senegal were found.

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 daily for 91 days were negative. Mitochondria and nuclei were ultrastructurally normal in appearance and internal structure.

In studies dating back as far as 1932, anaphylactic signs in guinea pigs injected with Acacia solution have been reported. In one of the studies, no signs of anaphylaxis were observed in rabbits injected with Acacia solution.

In rabbits and guinea pigs injected with 7% Gum Acacia solution, no deleterious effects on antibody production resulted. Effects on antibody production were judged by the development of agglutinative and complement-fixing activity in the serum to *Brucella abortus*.

Mouse footpad swelling test results indicated no 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.

Footpad swelling was considered indicative of a non-specific irritant effect in non-immunized male mice injected with Acacia Senegal. In another test, intradermal challenge after immunization of mice with Acacia Senegal 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. Slightly positive results for Gum Arabic in diploid human embryonic lung (WI-38) cells were noted in the cytogenetics assay. It was stated that further tests and a detailed statistical evaluation are needed in order to confirm this classification. The results of a subsequent study indicated that Gum Arabic induced chromosome aberrations in WI-38 human embryonic lung cells.

The mutagenicity of Gum Arabic was also evaluated in numerous *in vivo* assays, the results of which were mostly negative. Statistically significant results were noted in one of the three dominant lethal tests that were performed. It was noted that the biological significance of the positive finding could be questioned. Results were slightly positive in acute and short-term *in vivo* cytogenetics assays (rats); however, 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 four-week-old F344 rats (50 males, 50 females) and four to five-week-old $B6C3F_1$ mice (50 males, 50 females). 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 consecutive 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 non-carcinogenic 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.

At lower test concentrations, 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% or in the 1 to 5% concentration range (oral doses), failed to induce teratogenicity or other reproductive effects in female rats. Gum Arabic (5%) also did not cause abnormal sperm development in hamsters. Embryo-toxicity 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 (1 to 15 months) 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 six weeks. Gum Arabic was not detected in feces specimens collected from five male volunteers before or after administration of the gum. In this study, Gum Arabic (25 g in 125 ml 7% dextrose) was ingested daily over a period of 24 days. Marked increases in breath hydrogen production noted after ingestion were said to have been indicative of bacterial breakdown of Gum Arabic in the cecum and colon.

Toxic effects were not observed in five male subjects who ingested 25 g of Gum Arabic daily for 21 days.

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 four of the ten subjects indicated that Gum Arabic was the dominant gum antigen in two subjects. Cross reactivity between Gum Arabic and gum traga-canth was reported for a 24-year-old patient who developed sensitization to Quillaja bark (*Quillaja saponaria*) dust, which led to rhinitis and asthma.

A number of case reports on the allergenicity of

Gum Arabic have been identified in the published literature.

DISCUSSION

Section 1, paragraph (p) of the CIR Procedures states that "A lack of information about an ingredient shall not be enough to justify a determination of safety." In accordance with Section 30(j)(2)(A) of the Procedures, the Expert Panel informed the public of its decision that the data on Acacia Catechu, Acacia Concinna, Acacia Concinna Extract, Acacia Dealbata, Acacia Dealbata Extract, Acacia Decurrens, Acacia Decurrens Extract, Acacia Farnesiana, Acacia Farnesiana Extract, Acacia Senegal, Acacia Senegal Extract, and Acacia Senegal Gum Extract were insufficient to determine whether these ingredients, for purposes of cosmetic use, are either safe or unsafe. The Expert Panel released a 'Notice of Insufficient Data Announcement' on April 4, 1997 outlining the data needed to assess the safety of these ingredients. In the absence of information on the chemical constituents of the various Acacias, the following test data are needed on each of the ingredients in this review:

(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. Additionally, determine 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

(6) UV absorption spectrum; if there is significant absorbance in the UVA or UVB range, then a photosensitization study may be needed.

Note: Other studies may be requested after clarification of the chemical constituents of the Acacias

No offer to supply the data was received. In accordance with Section 45 of the CIR Procedures, the Expert Panel will issue a Final Report - Insufficient Data. When the requested data are available, the Expert Panel will reconsider the Final Report in accordance with Section 46 of the CIR Procedures, Amendment of a Final Report.

CONCLUSION

The Expert Panel concludes that the available data are insufficient to support the safety of Acacia Catechu, Acacia Concinna, Acacia Concinna Extract, Acacia Dealbata, Acacia Dealbata Extract, Acacia Decurrens, Acacia Decurrens Extract, Acacia Farnesiana, Acacia Farnesiana Extract, Acacia Senegal, Acacia Senegal Extract, and Acacia Senegal Gum Extract for use in cosmetic products.

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