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Final Report of the Safety Assessment of Acacia Catechu Gum, Acacia Concinna Fruit Extract, Acacia Dealbata Leaf Extract, Acacia Dealbata Leaf Wax, Acacia Decurrens Extract, Acacia Farnesiana Extract, Acacia Farnesiana Flower Wax, Acacia Farnesiana Gum, Acacia Senegal Extract, Acacia Senegal Gum, and Acacia Senegal Gum Extract[1]

These ingredients are derived from various species of the acacia plant. Only material derived from *Acacia senegal* are in current use according to industry data. The concentration at which these ingredients are reported to be used ranges from 9% in mascara to 0.0001% in tonics, dressings, and other hair-grooming aids. Gum arabic is a technical name for Acacia Senegal Gum. Gum arabic is comprised of various sugars and glucuronic acid residues in a long chain of galactosyl units with branched oligosaccharides. Gum arabic is generally recognized as safe as a direct food additive. Little information is available to characterize the extracts of other Acacia plant parts or material from other species. Acacia Concinna Fruit Extract was generally described as containing saponins, alkaloids, and malic acid with parabens and potassium sorbate added as preservatives. Cosmetic ingredient functions have been reported for Acacia Decurrens Extract (astringent; skin-conditioning agent—occlusive) and Acacia Farnesiana Extract (astringent), but not for the other Acacias included in this review. Toxicity data on gum arabic indicates little or no acute, short-term, or subchronic toxicity. Gum arabic is negative in several genotoxicity assays, is not a reproductive or developmental toxin, and is not carcinogenic when given intraperitoneally or orally. Clinical testing indicated some evidence of skin sensitization with gum arabic. The extensive safety test data on gum arabic supports the safety of Acacia Senegal Gum and Acacia Senegal Gum Extract, and it was concluded that these two ingredients are safe as used in cosmetic formulations. It was not possible, however, to relate the data on gum arabic to the crude Acacias and their extracts from species other than *Acacia senegal*. Therefore, the available data were considered insufficient to support the safety of Acacia Catechu Gum, Acacia Concinna Fruit Extract, Acacia Dealbata Leaf Extract, Acacia Dealbata Leaf Wax, Acacia Decurrens Extract, Acacia Farnesiana Extract, Acacia Farnesiana Flower Wax, Acacia Farnesiana Gum, and Acacia Senegal Extract in cosmetic products. The additional data needed to complete the safety assessment for these ingredients include (1) concentration of use; (2) identify the specific chemical constituents, and clarify the relationship between crude Acacias and their extracts and the Acacias and their extracts that are used as cosmetic ingredients; (3) data on contaminants, particularly relating to the presence of pesticide residues, and a determination of whether Acacia melanoxylon is used in cosmetics and whether acamelin (a quinone) and melacacidin (a flavin) are present in the Acacias that are being used; (4) skin sensitization study (i.e., dose response to be determined); (5) contact urticaria study at use concentration; and (6) ultraviolet (UV) absorption spectrum; if there is significant absorbance in the UVA or UVB range, then a photosensitization study may be needed. It was also noted that other data may be needed after clarification of the chemical constituents of the Acacia-derived ingredients.

INTRODUCTION

The Cosmetic Ingredient Review (CIR) Expert Panel began developing a safety assessment of Acacia-derived ingredients in 1996. In 1998, a final safety assessment was issued with the conclusion that the available data were not sufficient to support the safety of these ingredients in cosmetics. The needed data included concentration of use; specific chemical constituents, including the relationship between crude Acacias and their extracts and the Acacias and their extracts that are used as cosmetic ingredients; contaminants, particularly relating to the presence of pesticide residues and a determination if Acacia melanoxylon is used in cosmetics, and whether acamelin (a quinone) and melacacidin (a flavin) are present in the Acacias that are being used; skin sensitization dose response; contact urticaria data use concentration; and ultraviolet (UV) absorption; if there is significant absorbance in the UVA or UVB range, then a photosensitization study may be needed. The Panel noted that other studies may be requested after clarification of the chemical constituents of the Acacias.

In 2000 and 2001, new data were received including use concentration data on Acacia Senegal and Acacia Senegal Extract;

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[1]Reviewed by the Cosmetic Ingredient Review Expert Panel. This report was prepared by Wilbur Johnson, Senior Scientific Analyst/Writer.

information on the composition of gum arabic and various Acacia species; UV spectral analyses on Acacia Senegal Gum and Acacia Concinna Fruit Extract; impurities analysis for pesticide residues in gum arabic; human skin tolerance test (skin irritation evaluated) on 2% Acacia Concinna Fruit Extract; and human maximization test data on a mascara containing 8% Acacia Senegal. Based on this new information, the Panel has prepared this amended safety assessment.

The terminology with which the cosmetics industry describes these ingredients has changed over the past several years. Table 1 shows the progression of terminology from the mid-1990s to 2004. In some cases (e.g., Acacia Concinna Fruit Extract, Acacia Dealbata Leaf Wax, and Acacia Farnesiana Gum) the current name for the ingredient (Gottschalck and McEwen 2004) better reflects the source of the plant material. The current terminology will be used in this report.

A key factor in the determination that the current data are sufficient was the finding that gum arabic is the equivalent of Acacia Senegal Gum. Accordingly, the following is background information on gum arabic and its relationship to gum produced by the *Acacia senegal* plant.

Sudan is the world's largest producer of gum arabic, and it is the main source of gum in international trade. Nigeria is the second largest producer of gum arabic. In the Sudan, the term gum arabic is inclusive of two types of gum that are produced and marketed, "hashab" (from *Acacia senegal*) and "talha" (from *Acacia seyal*). Gum arabic (hashab) from the Sudan is considered to be of the highest quality, and sets the standard by which other "gum arabics" are judged. *Acacia senegal* intrinsically produces a high-quality exudate (pale to orange-brown-colored solid) with superior technical performance; and, in the Sudan, the collection, cleaning, sorting, and handling of it up to the time of export is well organized and highly efficient. In a wider sense, the name gum arabic often is understood to mean the gum from any *Acacia* species and is sometimes referred to as "Acacia gum." For example, gum arabic from Zimbabwe is derived from *Acacia karroo* (Food and Agriculture Organization of the United Nations 1999). Acacia Senegal Gum has been described as the major commercial Acacia gum (Anderson 1988).

Although most internationally traded gum arabic comes from *Acacia senegal*, the term "gum arabic" may not imply a particular botanical source. In a few cases, so-called gum arabic may not even have been collected from Acacia species, but may originate from *Combretum*, *Albizia*, or some other genus (Food and Agriculture Organization of the United Nations 1999).

In the *International Cosmetic Ingredient Dictionary and Handbook*, gum arabic is listed as a technical name for Acacia Catechu Gum, Acacia Farnesiana Gum, and Acacia Senegal Gum (Gottschalck and McEwen 2004). However, since this publication, the Cosmetic, Toiletry, and Fragrance Association (CTFA) determined that gum arabic does not apply to Acacia Catechu Gum or Acacia Farnesiana Gum and will no longer be listed in the *International Cosmetic Ingredient Dictionary and Handbook* as a technical/other name for these ingredients (CTFA 2000b).

According to CTFA, gum arabic applies to the dried gummy exudate from branches and stems of *Acacia senegal* and other Acacia species from Africa, and *Acacia catechu* and *Acacia farnesiana* are not African species (CTFA 2000b).

This definition is similar to the following definition of Acacia that is found in the *National Formulary* (United States Pharmacopeial Convention 2000): Acacia is the dried gummy exudate from the stems and branches of *Acacia senegal* (Linné) Wildenow or of other related African species of Acacia (Family Leguminosae). It has also been described as a complex mixture of calcium, magnesium, and potassium salts of arabic acid. Arabic acid is a complex of galactose, rhamnose, arabinose, and glucuronic acid (Frutarom Meer Corporation, no date).

Gum arabic is a substance that is generally recognized as safe (GRAS) for direct addition to human food under the provisions of Section 184.1330 of the Code of Federal Regulations (21 CFR 184.1330). A report, prepared for the Food and Drug Administration (FDA), summarizing all available scientific data (1920 to 1972) related to the safety of gum arabic as a food ingredient has been published (Informatics Inc. 1972). Studies from that report are referenced in the text of this report.

In a subsequent report (prepared for FDA) evaluating the safety of gum arabic as a food ingredient, the Select Committee on GRAS Substances of the Life Sciences Research Office, Federation of American Societies for Experimental Biology (FASEB), concluded that "there is no evidence in the available information on gum arabic that demonstrates a hazard to the public when it is used at levels that are now current and in the manner now practiced. However, it is not possible to determine, without additional data, whether a significant increase in consumption would constitute a dietary hazard" (FASEB 1973).

The Select Committee also determined that additional experiments should be undertaken to evaluate the significance of gum Arabic allergenicity to the population as a whole, and that it may be advisable to conduct feeding studies in several animal species (including pregnant animals) at dosage levels that approximate and exceed the current maximum daily human intake (see "Noncosmetic Use").

Studies from the 1973 FASEB report are summarized in the text of this report. Studies on Acacia Senegal Gum and other species of Acacia (listed in the *International Cosmetic Ingredient Dictionary and Handbook* and those not listed) that have been published since the FASEB report was issued are also included. To ensure that the information in the present report is representative of the published chemistry and toxicity data on species of Acacia, the data presented involve various parts/components of the Acacia tree as well as the gummy exudate.

CHEMISTRY

Definitions of various ingredients derived from Acacia species in the *International Cosmetic Ingredient Dictionary and*

TABLE 1
Acacia-derived cosmetic ingredient terminology, description, and function

1995–1997 Terminology (Wenninger and McEwen 1995, 1997)			2004 Terminology (Gottschalck and McEwen 2004)		
Name	Description	Cosmetics function	Name	Description	Cosmetics function
Acacia Catechu	Plant material derived from *Acacia catechu*	Biological additive	Acacia Catechu	EU term for Acacia Catechu Gum	N/A
Acacia Catechu	Dried, crushed core of *Acacia catechu*	Biological additive	Acacia Catechu Gum	Dried, crushed core of *Acacia catechu*	Not reported
Acacia Concinna	Plant material derived from *Acacia concinna*	Not reported	Acacia Concinna	EU term for Acacia Concinna Fruit Extract	N/A
Acacia Concinna Extract	Extract of the fruit of *Acacia concinna*	Biological additive	Acacia Concinna Fruit Extract	Extract of the fruit of *Acacia concinna*	Not reported
Acacia Dealbata	Plant material derived from *Acacia dealbata*	Not reported	Acacia Dealbata	EU term for Acacia Dealbata Leaf Extract	N/A
Acacia Dealbata Extract	Extract of the leaves of *Acacia dealbata*	Biological additive	Acacia Dealbata Leaf Extract	Extract of the leaves of the wattle, *Acacia dealbata*	Not reported
			Acacia Dealbata Leaf Wax	Wax obtained from the leaves of *Acacia dealbata*	Skin-conditioning agent—emollient; skin protectant
Acacia Decurrens	Plant material derived from *Acacia decurrens*	Not reported	Acacia Decurrens	EU term for Acacia Decurrens Extract	N/A
Acacia Decurrens Extract	Extract of the acacia, *Acacia decurrens*	Biological additive	Acacia Decurrens Extract	Extract of the acacia, *Acacia decurrens*	Astringent; Skin-conditioning agent—Occlusive
Acacia Farnesiana	Plant material derived from *Acacia farnesiana*	Not reported	Acacia Farnesiana	EU term for Acacia Farnesiana Extract, Flower Wax, and Gum	N/A
Acacia Farnesiana	Plant material derived from the dried, gummy exudate of the acacia, *Acacia farnesiana*	Not reported	Acacia Farnesiana Gum	Plant material derived from the dried, gummy exudate of the acacia, *Acacia farnesiana*	Not reported
Acacia Farnesiana Extract	Extract of the flowers and stems of the the acacia, *Acacia farnesiana*	Biological additive	Acacia Farnesiana Extract	Extract of the flowers and stems of the the acacia, *Acacia farnesiana*	Astringent
			Acacia Farnesiana Flower Wax	wax obtained from the flowers of *Acacia farnesiana*	Skin protectant
Acacia Senegal	Plant material derived from *Acacia senegal*	Not reported	Acacia Senegal	EU term for Acacia Senegal Extract, Gum, and Gum Extract	N/A

(Continued on next page)

TABLE 1
Acacia-derived cosmetic ingredient terminology, description, and function *(Continued)*

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

Handbook are included in Table 1 (Gottschalck and McEwen 2004). CAS numbers are listed for the following two: Acacia Catechu Gum (CAS no. 8001-76-1) and Acacia Senegal Gum (CAS no. 9000-01-5).

Chemical and Physical Properties

Gum Arabic

The gummy exudate from the *Acacia senegal* is a proteinaceous polysaccharide, with protein content ranging from approximately 1.5% to 3% for samples from various producing areas (World Health Organization 1990).

Gum arabic is a white powder that is readily soluble in water, but insoluble in alcohol (Anonymous 1993). Molecular weights of ∼850,000 (Ross et al. 1984a, 1984b) and ∼240,000 (Frutarom Meer Corporation no date), and a density of 1.35 to 1.49 (Dangerous Properties of Industrial Materials Report 1981) have been reported. An aqueous solution is acid to litmus (Lewis 1993a).

Pazur et al. (1986) indicated that gum arabic is composed of D-galactose, L-rhamnose, L-arabinose, and D-glucuronic acid residues in an arrangement of a main chain of galactosyl units joined by β-D-(1 → 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. Based on methylation and degradation studies of gum arabic (*Acacia senegal*) along with periodate oxidation and other confirmatory reactions, the structure for gum arabic shown in Figure 1 was proposed (Informatics Inc. 1972).

Gum arabic is almost completely soluble in twice its weight of cold water, and the viscosity of the gum increases slowly at concentrations up to 25%. At concentrations greater than 25%, the viscosity increases much more rapidly in proportion to the gum content (Frutarom Meer Corporation no date).

UV Absorption

An increase in absorbance for Acacia Senegal was observed between 400 nm and approximately 260 nm, reaching a plateau at wavelengths ranging from 270 to ∼250 nm. A rapid increase in absorbance was observed at wavelengths less than 250 nm (Avon Products, Inc. 2000a). UV absorption spectra provided on two other lots of Acacia gum (Acacia Senegal) were both similar to the preceding UV spectral analysis (Avon Products, Inc. 2000b).

Methods of Production

Gum arabic is produced when the Acacia tree is stressed by infection, poor nutrition, heat, or lack of moisture. The gum exudes through wounds in the bark that occur naturally or are purposely made to stimulate production. The exudate dries rapidly, is collected as hardened drops or tears, sorted, graded, and marketed. The gum becomes harder during storage; market preferences exist for both the harder (old) and softer (new) gum (FASEB 1973).

According to another source, the removal of the bark that adheres to the tears is critical to the production of quality gum arabic. Additionally, in order to produce quality products, elaborate processes for the preclearing of milled gum and the centrifugation and filtration of feed solutions for spray dried gum must be followed. The major growing regions for gum arabic are in the Sudan and West Africa, and the Kordofan grade is considered the best (Frutarom Meer Corporation, no date).

Gum arabic in solid form is imported from the Sudan. According to one source, the solid is converted to a liquid form and

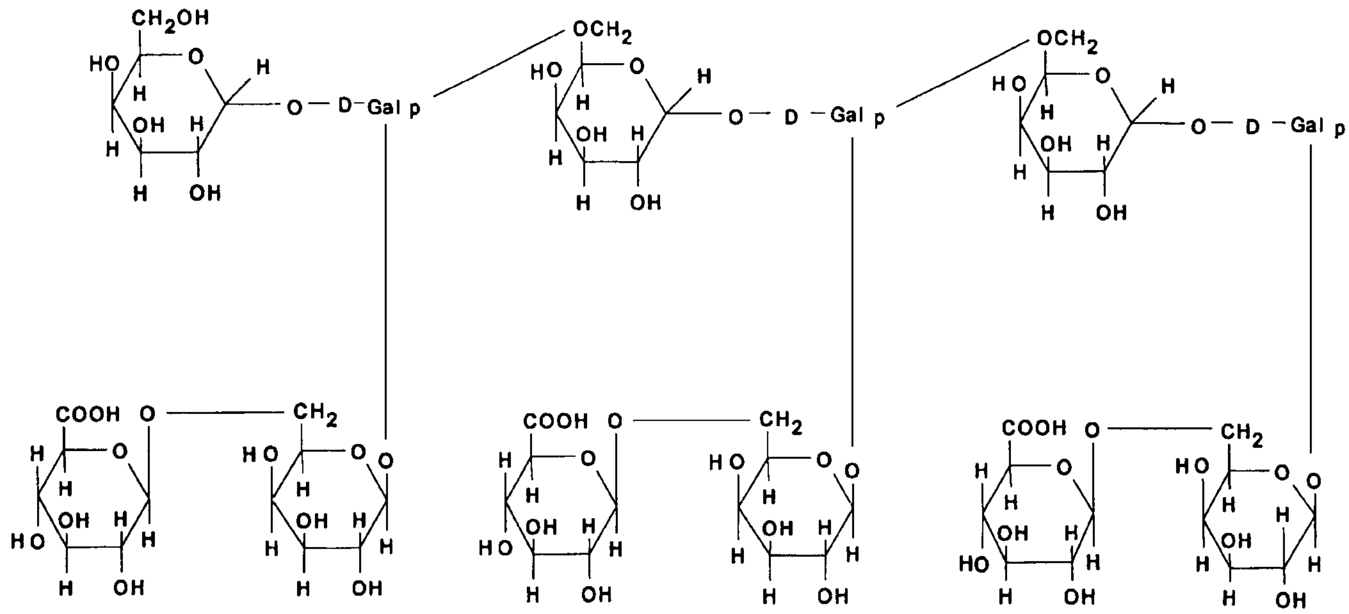

FIGURE 1
Proposed structure for Gum Arabic (Informatics 1972).

the preservatives Proxel GXL (0.13%) and sodium benzoate are then added. Proxel GXL consists of 20% 1,2-benzisothiazolin-3-one (BIT) in aqueous dipropylene glycol (Freeman 1984).

Crude Acacia Concinna results from the drying and pulverization of the pods of *Acacia concinna*. The extract of these pods (Acacia Concinna Fruit Extract) is drawn by cold processing (Carlisle International Corporation 1997a).

Composition, Analytical Methods, and Impurities

The following three grades of gum arabic have been noted in the published literature: (1) processed gum arabic recovered by spray-drying from a solution of commercial food grade gum arabic after filtration to remove sand, etc., and after heat treatment to effect pasteurization; (2) finely powdered natural gum arabic of poor commercial quality, giving solutions of a dark reddish brown color; (3) finely powdered natural gum arabic of very high quality, giving essentially colorless solutions (Strobel et al. 1982). Gum arabic has been analyzed by gas chromatography (Lawrence and Iyengar 1985) and has been identified by microelectrophoresis (Informatics Inc. 1972).

Powdered gum arabic contains moisture (15% maximum) and insoluble ash (0.5% maximum). The pH of a 10% solution is approximately 4.6 (Frutarom Meer Corporation, no date). The following specifications exist for United States Pharmacopoeia (USP) grade Acacia: loss on drying (15% maximum), total ash (4% maximum), arsenic (3 ppm), lead (0.001%), and heavy metals (0.004%) (United States Pharmacopeial Convention, Inc. 2000).

FDA has listed Acacia (Gum Arabic) as a direct food additive that meets the specifications of the *Food Chemicals Codex* (21 CFR 184.1330). The specifications for food grade Acacia include arsenic (3 mg/kg maximum); ash, acid-insoluble (0.5% maximum); ash, total (4% maximum); heavy metals (0.002% maximum); insoluble matter (1% maximum); lead (5 mg/kg maximum); and loss on drying (15% maximum) (Food Chemicals Codex 1996).

Anderson et al. (1990) compared the amino acid composition of Sudanese and Nigerian gum arabic. Analyses were done on samples collected over a 13-year period for samples from the Sudan and over 9 years for those from Nigeria. The data are presented in Table 2.

Anderson et al. (1991) analyzed gum arabic samples provided by importers shown in Table 3. All samples conformed to the revised Joint Food and Agriculture Organization of the United Nations/World Health Organization, Expert Committee on Food Additives (JECFA) specification in respect of solubility, complete in cold water; acid-insoluble ash, (>0.5%) and matter (>1%); starch/dextrin (absent); tannin (absent); arsenic (>3 ppm), lead (>10 ppm), heavy metals (>40 ppm) (JECFA 1990). All samples were confirmed by nuclear magnetic resonance (NMR) spectroscopy to be "good" *Acacia senegal*.

Anderson et al. (1990) also analyzed the ash from gum arabic samples from these same two countries. These data are given in Table 4.

West Coast Analytical Service, Inc. (1999) analyzed gum arabic for pesticide residues using USP methodology and found no detectable pesticide residues.

Other food additive specifications for gum arabic from the Food and Agriculture Organization (FAO) of the United Nations defined the material as a dried exudate obtained from the stems and branches of *Acacia senegal* (L.) Willdenow or *Acacia seyal*

TABLE 2
The amino acid composition of Sudanese and Nigerian Gum Arabic (*Acacia senegal*) (Anderson et al. 1990)

Amino acid	Sudanese samples Mean residues/1000 residues (13 years total between 1904 and 1989)	Nigerian samples Mean residues/1000 residues (9 years total between 1905 and 1967)
Alanine	27 ± 3	24 ± 4
Arginine	13 ± 4	12 ± 1
Aspartic acid	68 ± 13	61 ± 16
Cystine	2 ± 4	0
Glutamic Acid	42 ± 10	42 ± 15
Glycine	50 ± 5	50 ± 6
Histidine	44 ± 8	48 ± 5
Hydroxyproline	304 ± 47	331 ± 73
Isoleucine	12 ± 3	13 ± 3
Leucine	66 ± 7	69 ± 8
Lysine	25 ± 3	24 ± 6
Methionine	2 ± 2	1
Phenylalanine	33 ± 5	29 ± 10
Proline	63 ± 14	55 ± 9
Serine	129 ± 11	129 ± 13
Threonine	68 ± 9	67 ± 8
Tyrosine	14 ± 5	14 ± 4
Valine	35 ± 8	32 ± 6

(family Leguminosae), include the following: loss on drying (not more than 15% [105°C, 5 h] for granular and not more than 10% [105°C, 4 h] for spray dried material); total ash (not more than 4%), acid-insoluble ash (not more than 0.5%), acid-insoluble matter (not more than 1%); and lead (not more than 2 mg/kg) (FAO 1999).

TABLE 4
Cation composition of ash from Sudanese and Nigerian Gum Arabic (*Acacia senegal*) (Anderson et al. 1990)

Cation	Sudanese samples Mean μg/g ash unless expressed as ppm (15 years total between 1904 and 1989)	Nigerian samples Mean μg/g ash unless expressed as ppm (9 years total between 1905 and 1967)
Aluminum	190 ± 53	311 ± 156[a]
Calcium	256,000 ± 34,000	316,000 ± 56,000
Chromium	47 ± 22	34 ± 26
Copper	52 ± 27	66 ± 65[b]
Iron	128 ± 84	110 ± 33
Lead	6 ± 2	11 ± 7
Magnesium	38,000 ± 15,000	39,000 ± 15,000
Manganese	100 ± 95	57 ± 27
Nickel	10 ± 11	12 ± 17
Potassium	237,000 ± 37,000	221,000 ± 43,000
Sodium	9,400 ± 4,480	10,200 ± 5,200
Zinc	24 ± 10	40 ± 49[c]
Arsenic	<1 ppm	<1 ppm
Cadmium	<1 ppm	<1 ppm
Cobalt	<1 ppm	<1 ppm
Molybdenum	<1 ppm	<1 ppm

[a]Mean = 266 (n = 8) if one value, 675, is treated as an outlier.
[b]Mean = 47, if one value, 225, is treated as an outlier.
[c]Mean = 25, if one value, 159, is treated as an outlier.

Acacia Concinna Fruit Extract

Acacia Concinna Fruit Extract consists of 1 part of extract obtained from 1 part of dry pods of *Acacia concinna*. It contains the active constituents of the pods of *Acacia concinna*, such as

TABLE 3
Analytical data for natural Gum Arabic samples provided by importers in 1990/1991 (Anderson et al. 1991)

Sample no.	Sample sent by	Date received	% H_2O[a]	% Ash[a]	% N[a]	Specific rotation (degrees)
N1	American importer A	12/90	13.2	3.8	0.34	−29
N2	American importer A	12/90	13.9	4.0	0.36	−31
N3	Italian importer B	12/90	14.4	3.3	0.31	−30
N4	British importer C	12/90	14.5	3.6	0.37	−31
N5	British importer D	12/90	12.2	3.5	0.35	−33
N6	British importer E	12/90	14.9	2.0	0.38	−33
N7	German importer A	1/91	14.4	4.0	0.26	−34
N8	American importer G	1/91	15.0	3.2	0.29	−26
N9	American importer H	1/91	13.9	3.9	0.33	−32
N10	British importer K	2/91	13.3	3.7	0.34	−28
N11	Italian importer L	2/91	14.8	3.4	0.30	−29
Mean values			14.0	3.6	0.33	−30.5

[a]Dry-weight basis, as specified (Food and Agriculture Organization of the United Nations 1990).

TABLE 5
Acacia Concinna Fruit Extract specifications (Carlisle International Corporation 1997a)

Specification	Standard
Color	Brown
pH	4 to 6
Specific gravity (at 25°C)	1.0 to 1.10
Refractive index (at 20°C)	1.1 to 1.4
Dried residue (2 h/110°C)	10% to 20%
Water	60% to 65%
Propylene glycol	35% to 40%
Water solubility	Soluble
Preservatives	Parabens and potassium sorbate
Heavy metal	<10 ppm
UV/VIS spectrophotometry	
Absorbance at 220 nm of a 0.1% aqueous solution	1.0 ± 0.25
Absorbance at 220 nm of a 0.2% aqueous solution	2.0 ± 0.20
Other constituents (HPTLC method)	Saponins, alkaloids, malic acid
Maximum total bacterial count	100/g
Maximum yeasts and molds	0/g

vegetable saponins. The raw material (*Acacia concinna*) from which Acacia Concinna Fruit Extract is derived is from wild, crafted sources free of contamination with pesticide residues. The standard analytical profile of Acacia Concinna Fruit Extract is given in Table 5. A sample "passes" if it meets these specifications (Carlisle International Corporation 1997a).

Information on the composition and impurities of various species of Acacia and their contaminants is included in Table 6. The Acacia species that are listed in the *International Cosmetic Ingredient Dictionary and Handbook* are identified with an asterisk.

As noted in Table 6, aflatoxin has been detected in the bark and seeds of *Acacia catechu* (Roy and Kumari 1988, 1991). Abdalla (1988) also described gum-yielding Acacia twigs from the Sudan (supplier of Acacia Senegal Gum) as a source of aflatoxin (81 to >1000 μg/kg).

Smith et al. (1990), however, found no detectable aflatoxin in either of two samples of gum arabic analyzed using an enzyme-linked immunosorbent assay. The assay system was capable of determining aflatoxin in the concentration range of 2.0 to 200.0 ppb in gum arabic.

Data from the European Federation for Cosmetic Ingredients (EF*f*CI) describes the components of the plant material from various Acacia species. While there are some similarities, there are many differences in composition (EF*f*CI 2000). These data are given in Table 7.

Reactivity

When Gum Acacia is weakly hydrolyzed by hydrochloric acid at room temperature, pentose is split off (Marrack and Carpenter 1938). Partial acid hydrolysis has also yielded galactose and complex sugar acids (Heidelberger et al. 1929).

Gum Acacia emits acrid smoke when heated to decomposition (Lewis 1993b). Heating a solution of Acacia for a few minutes at 100°C destroys peroxidase (oxidizing agent) present in the gum and the colored derivatives produced (Gennaro 1990).

USE

Purpose in Cosmetics

The functions of these ingredients in cosmetics as described in the *International Cosmetic Ingredient Dictionary and Handbook* are given in Table 1 (Wenninger et al. 2000).

Reportedly, *Acacia concinna* pods are a useful hair wash, in that they promote hair growth, kill lice, and remove dandruff. The active constituents of *Acacia concinna* pods (saponins, alkaloids, tannins, and malic acid) are said to have cleansing, stimulating, and astringent properties. The astringent action provides toning of the scalp and conditioning of the hair. Additionally, the active constituents are said to offer effective skin and scalp exfoliation (Carlisle International Corporation 1997b).

Scope and Extent of Use in Cosmetics

The product formulation data submitted to the FDA in 2001 indicated that Acacia was used in 33 cosmetic products and that Acacia Senegal was used in 1 cosmetic product (Table 8) (FDA 2001). Neither the species nor the plant part was further delineated in the category "Acacia." It is assumed that Acacia Senegal is Acacia Senegal Gum.

Current concentration of use data are given in Table 8. These data from industry (CTFA 2000a) show the highest concentration of Acacia Senegal Gum (9%) in shampoos. Acacia Senegal Gum Extract was reported at a concentration of 0.001% in bath soaps and detergents. For many uses of these ingredients, information regarding use concentration for specific product categories is provided, but the number of such products is not known, but they must be assumed to be in use.

Recommended use concentrations of Acacia Concinna Fruit Extract are 0.5% to 5.0% (Carlisle International Corporation 1997a) and 1.0% to 2.0% for use in shampoos, hair packs, hair conditioners, and hair rinses (Carlisle International Corporation 1997b).

Cosmetic products containing Acacia are applied to most parts of the body and could come in contact with the ocular and nasal mucosae. These products could be used on a daily basis, and could be applied frequently over a period of several years.

Acacias are not included among the substances listed as prohibited from use in cosmetic products that are marketed in the European Union (EEC 2001). The European Union terminology

TABLE 6
Composition and impurities data on Various Species of Acacia

Acacia species (part/source)	Analytical method	Components	Reference
Acacia atramentaria and *Acacia tortuosa* (leaves)	Gas chromatography and NMR spectroscopy	Proacacipetalin (cyanogenic glucoside)	Seigler et al. 1983
Acacia albida, Acacia ataxa-cantha, Acacia catechu, Acacia confusa, Acacia coulteri, Acacia erubescens, Acacia ferruginea, Acacia galpinii, Acacia hamulosa, Acacia mellifera, Acacia modesta, Acacia nigrescens, Acacia polyacantha, Acacia rovumae, Acacia senegal*, Acacia venosa*, and *Acacia welwitschii* (seeds)	Ion exchange chromatography	α-Amino-β-oxalylaminopropionic acid (neurotoxic lathyrogen)	Quereshi et al. 1977
Acacia aroma (leaves)	Gas chromatography and NMR spectroscopy	Linamarin and lotaustralin (cyanogenic glucosides)	Seigler et al. 1983
*Acacia catechu** (seed)	Thin-layer chromatography and spectrophotometry	Aflatoxin B_1 (0.01 to 0.76 μg/g)	Roy and Kumari, 1991
*Acacia concinna** (pods)	—	Highly polar saponin mixture. Hydrolysis with alkali yields 5 triterpenoidal prosapogenols (concinnosides A, B, C, and D), 4 glycosides (acadiaside, julibroside A1, julibroside A3, albiziasaponin C), and aglycone, acacic acid lactone	Abul et al. 1997
*Acacia concinna** (fruit)	—	Kinmoonsides A–C (3 cytotoxic saponins)	Tezuka et al. 2000
*Acacia farnesiana** (pod, leaf, stem, old stem, and flower)	Phytochemical screening	Carbohydrates and/or glycosides, reducing sugars, hydrolyzable tannins, alkaloids and nitrogenous bases, unsaturated sterols, and/or terpenes, and coumarins (all organs)	Wassel et al. 1992
*Acacia farnesiana** (pod, leaf, old stem, and flower)	—	Flavonoids (all organs except stem)	Wassel et al. 1992
*Acacia farnesiana** (pod, leaf, stem)	—	Cyanogenic glycosides (in pod, leaf, and stem)	Wassel et al. 1992
*Acacia farnesiana** (flower)	—	Volatiles (flower)	Wassel et al. 1992
*Acacia farnesiana** oil	Thin layer chromatography	Anisaldehyde, benzalcohol, benzaldehyde, cuminicalcohol, farnesol, cuminicaldehyde, geraniol, geranyl acetate, ionone, linalool, linalyl acetate, nerolidol, terpineol, and methyl salicylate	El-Hamid and Sidrak 1970
*Acacia farnesiana** (leaves)	—	Total soluble phenols ranged from 10.27% to 35.46%. Condensed tannins ranged from 0.5% to 8.28% on dry matter basis	Sotohy et al. 1995
*Acacia farnesiana** (leaves)	—	Cyanogenic glycoside (linamarin or lotaustralin may be present)	Secor et al. 1976

(Continued on next page)

TABLE 6
Composition and impurities data on Various Species of Acacia *(Continued)*

Acacia species (part/source)	Analytical method	Components	Reference
Acacia tortilis (gum and bark extracts)	High-performance liquid chromatography	Smooth muscle relaxants: quaracol A and B (in gum) and (+)-fisetinidol (in gum and bark)	Hagos and Samuelson 1988
Acacia georginae (seeds)	Extractive and chromatographic procedures	Fluoroacetic acid	Oelrichs and McEwan 1962
Acacia globulifera (leaves)	Gas chromatography and NMR spectroscopy	Epiproacacipetalin (cyanogenic glucoside)	Seigler et al. 1983
Acacia modesta (stem bark, heartwood, and leaf extracts)	Thin-layer chromatography	α-amyrin, betulin, octacosanol and ε-sitosterol (in stem bark); γ-sitosterol and pinitol (in heartwood); octacosane, hentriacontane, octacosanol, and hentriacontanol (leaves)	Joshi et al. 1975
*Acacia mollissima, Acacia confusa, Acacia longifolia, Acacia decur-rence**, *Acacia dealbata**, *Acacia baileyana*, and *Acacia verticillata* (leaves)	Amino acid autoanalyzer used	(−)-*trans*-4-hydroxypipecolic acid	Marakesh et al. 1969

*The Acacia species listed in the *International Cosmetic Ingredient Dictionary.*

for these ingredients is described in Table 1, where the genus and species names are used to describe all of the plant material (e.g., gum, extract, etc.) derived from that particular genus/species, independent of the plant part from which the material is derived.

The Acacias reviewed in this report are not included on the list of ingredients that must not be combined in cosmetic products that are marketed in Japan (Ministry of Health, Labor and Welfare [MHLW] 2000a) or on the list of restricted ingredients for cosmetic products that are marketed in Japan (MHLW 2000b).

Noncosmetic Use

Gum arabic is a substance that is generally recognized as safe (GRAS) for direct addition to human food under the provisions of Section 184.1330 of the Code of Federal Regulations (CFR). It is approved for use in various food categories at the following maximum permitted usage levels: 2.0% (beverage and beverage bases), 5.6% (chewing gum), 12.4% (confections and frostings), 1.3% (dairy product analogs), 1.5% (fats and oils), 2.5% (gelatins, puddings, and fillings), 46.5% (hard candy and cough drops), 8.3% (nuts and nut products), 6.0% (quiescently frozen confection products), 4.0% (snack foods), 85.0% (soft candy), and 1% (all other food categories).

Uses of gum arabic in the various food categories include: emulsifier and emulsifier salt, flavoring agent and adjuvant, formulation aid, stabilizer and thickener, humectant, surface-finishing agent, processing aid, and texturizer (21 CFR 184.1330). Gum arabic is also listed as one of the optional blending ingredients of vanilla powder (21 CFR 169.179) and vanilla-vanillin powder (21 CFR 169.182).

The following maximum values for possible daily human intake (g/kg body weight) of gum arabic in the total diet have been calculated for various age groups by the Select Committee on GRAS Substances using data from the National Research Council: 115 mg/kg (0 to 5 months), 322 mg/kg (6 to 11 months), 329 mg/kg (12 to 23 months), and 113 mg/kg (2 to 65 + years) (FASEB 1973).

At the 35th meeting of the JECFA, held in Rome from May 29 to June 7, 1989, JECFA confirmed its acceptable daily intake (ADI) of gum arabic as "not specified." Here, gum arabic (a.k.a. gum Acacia) is defined as the dried gummy exudate from tropical and subtropical *Acacia senegal* trees.

ADI "not specified" is applicable to a food substance of very low toxicity, which, on the basis of the available data (chemical, biochemical, toxicological, and other), the total dietary intake of the substance arising from its use at the levels necessary to achieve the desired effect, and from its acceptable background in food does not, in the opinion of the JECFA, represent a hazard to health. For that reason, and for reasons stated in individual evaluations, the establishment of an ADI expressed in numerical form is not deemed necessary. An additive meeting this criterion must be used within the bounds of good manufacturing practice, i.e., it should be technologically efficacious and should be used at the lowest level necessary to achieve this effect; it should not conceal inferior food quality or adulteration, and it should not create a nutritional imbalance (JECFA 1990).

TABLE 7
Chemicals found in Acacia species (EF*f*CI 2000)

Acacia catechu plant	Acacia decurrens plant	Acacia farnesiana plant	Acacia senegal plant
(+)-afzelichin	(+)-catechin	(+)-catechol	4-methoxyglucuronic acid
3-(beta-L-arabopyranoside)-L-arabinose	3,3′,4′,5′,7-pentahydroxy-2-phenylchroman	(+)-gallocatechol	Arabic acid
3-methoxyflavones	3,3′,4,4′,7′-pentahydroxyflavin	Apigenin-6,8-bis-beta-D-glucopyranoside	Arabinose
4-(4-*O*-methyl-alpha-D-glucuronoside)-L-arabinose	3-methyl-L-rhamnose	Aromadendrin	Ascorbic acid
4-hydroxypipecolic acid	7,3′,4′,5-tetrahydroxyflavan-3-O-L-catechin	Aspartic acid	Aspartic acid
5-(beta-D-xylopyranoside)-L-arabinose	Acetic acid	Cresols	Beta sitosterol
7,3,4-trihydroxy-3,8-dimethoxyflavone	Aldobionic acid	Ellagic acid	Beta sitosterol-D-glucose
7,8,14′-trihydroxyflavonol	Alpha cellulose	Ethyl ester	Cysteine
7,8,4-trihydroxy-3-methoxyflavone	Anthocyanidin	Hydroxyacetophenone	D-galactose
8-methoxyfisetin	Anthocyanilidine	Isorhamnetin-3-rutinoside	D-glucoside
9-methoxyflavone-3,4-diones	Beta carotene	Kaempferol	Dimethyltryptamine
Acacatechin	Carbohydrates	Kaempferol-7-galloylglucose	Erythrodiol
Acetaldehyde	Cellulose	Kaempferol-7-glucoside	Galactoglucuronid acid
Aldobiuronic acid	D-galacturonic acid	Linamarin	Glucuronic acid
Alpha-amino-beta-oxalylaminopropionic acid	D-pinetol	Lotaustralin	HCN
Alpha catechin	Fiber	*m*-digallic acid	Hentriacontane
Beta catechin	Fisetinidin	Methyl gallate	Hentriacontanol
Boron	Fructose	Mucilage	Kaempferol
Catechuic acid	Gallocatechin	Myricetin-4′-methylether-3-rhamnoside	L-arabinose
Catechutannic acid	Indoleacetic acid	*N*-acetyl-djenkkolic acid	L-rhamnose
Cobalt	L-arabinose	Naringenin-7-glucoside	Leucine
D-galactose	L-rhamnose	Naringenin-7-rhamnoglucoside	Magnesium
D-glucuronic acid	Lignin	Pipecholic acid	Octacosanol
D-xylose	Mearnsitrin	Prunin-*O*-6′-gallate	Peroxidase
Diamino acid	Methanol	Quercetin-3-*O*-rutinoside	Potassium
Dihydrokaempferol	Methylsalicylic ester	Salicylic acid	Quercetin
DL-catechol	Pelargonidin	Tyramine	Rhamnose
DL-epicatechin	Phlobaphene		Rhamnose hydrate
Fisetin	Phlobaphene anhydride		Serine
Flavotannin	Phloroglucinol		Sitosterol
Formaldehyde	Proanthocyanidin		Sodium

(Continued on next page)

TABLE 7
Chemicals found in Acacia species (EF*f*CI, 2000) *(Continued)*

Acacia catechu plant	Acacia decurrens plant	Acacia farnesiana plant	Acacia senegal plant
Gallic acid	Protocatechuic acid		Sucrose
Gallotannin	Robinetin		Tannin
Gamma-catechin	Rutin		Uronic acid
Glucosyluronic acid	Xanthophyll		Valine
Gum			
Isocacatechin			
Isorhamnetin			
Isovaleraldehyde			
Kaempferol			
L-epicatechin			
L-leucomacluricglycol ether			
L-rhamnose			
Magnesium			
Malate dehydrogenase			
Manganese			
Peroxidase			
Phlobatannin			
Phosphatase			
Procyanidin			
Quercetagetin			
Quercetin			
Quercitrin			
Rutin			
Silicon			
Tannin			
Taxifolin			
Uronic acid			

Gum arabic (Acacia Senegal Gum) is used in the pharmaceutical industry to stabilize emulsions during the preparation of tablets (Collins et al. 1987). It is also used for its demulcent action in the treatment of throat or gastric inflammation (Gennaro 1990).

The therapeutic efficacy of Acacia Catechu in the treatment of lepromatous leprosy has been reported (Ojha et al. 1969).

Gum Arabic has also been used in glues, lithographic solutions, and matches (tip and binder in striking surface), and polisher and textile finishes (van Ketel 1984).

The following uses of Acacia Concinna in folk medicine have been reported: A chutney (pungent relish of fruits, spices, and herbs) made of the tender leaves of *Acacia concinna*, salt tamarind, and chilies is administered for the treatment of bilious affections such as jaundice. An infusion of the leaves is used in the treatment of malarial fever; it checks flatulence and serves as a mild laxative. Furthermore, repeated, large doses of a decoction of the *Acacia concinna* pods act as an emetic and purgative (Carlisle International Corporation 1997b).

An ointment made from the *Acacia concinna* pods reportedly is used in the treatment of skin diseases (Carlisle International Corporation 1997b).

BIOLOGICAL PROPERTIES

Absorption, Distribution, Metabolism, and Excretion

Gum Arabic

The weight gain for rats fed gum arabic at a dietary concentration of 16% was 75% of that reported for control rats. It was determined that approximately 80% of the gum arabic was absorbed (Informatics 1972).

In a study using rats, an apparent decrease in the caloric value of gum arabic with increasing administered dose was noted. Gum arabic was incorporated into the diet at concentrations of 5%, 10%, and 17%. Digestibility data indicated that up to 80% of the gum arabic was absorbed (Informatics Inc. 1972).

Following a 48 h fast, 20 young male rats were fed 10 mg of a mixture consisting of 34% white, powdered gum arabic and 66% cacao butter. At 72 h after feeding, the rats were anesthetized

TABLE 8
Product formulation data on Acacia and Acacia Senegal

Product category (total formulations in category) (FDA 2001)	Formulations with ingredient (FDA 2001)	Concentration of use (CTFA 2000a) (%)
Acacia		
Other bath preparations (193)	1	—
Mascara (187)	18	—
Other eye makeup preparations (151)	2	—
Hair tints (49)	1	—
Hair color sprays (Aerosol) (5)	1	—
Other hair-coloring preparations (59)	3	—
Foundations (319)	1	—
Lipstick (942)	1	—
Other makeup preparations (186)	1	—
Body and hand skin care preparations (excluding shaving) (827)	3	—
Paste masks (mud packs) (269)	1	—
2001 totals for Acacia	33	—
Acacia Senegal Gum		
Eyebrow pencil	—	1
Eyeliner	—	3
Mascara (187)	1	3–9
Powders (dusting and talcum; excluding aftershave talc)	—	0.5
Tonics, dressings, and other hair grooming aids	—	0.0001
Other skin care preparations	—	0.02
2001 totals for Acacia Senegal Gum	1	—
Acacia Senegal Gum Extract		
Bath soaps and detergents	—	0.001
2001 totals for Acacia Senegal Gum Extract	—	—

and the liver was removed and analyzed for glycogen content. The difference in glycogen concentration between control and fed rats was insignificant. Therefore, it was concluded that the gum arabic molecule was not metabolized by enzymes of the rat digestive tract (Informatics Inc. 1972; FASEB 1973).

Other studies have indicated that gum arabic is partially digested in the rat. In one study, weight gain and feed efficiency were determined using groups of six rats fed 15% gum arabic for 62 days. Feed efficiency was identical between experimental and control groups. However, compared to the control group (mean weight gain = 199 g), rats fed gum arabic had a mean weight gain of 224 g. In another study, groups of five rats were pair-fed gum arabic (0.75 g/day; added to 5 g basal diet). Results indicated that the digestibility of gum arabic was 71% (Informatics Inc. 1972).

Ross et al. (1984b) evaluated the metabolism of gum arabic using albino Wistar male rats (3 months old; weights = 350 g). The number of animals used in the study was not stated. Two groups of animals were fed Oxoid breeders diet only and Oxoid breeders diet plus 200 g gum arabic/kg ad libitum, respectively, for 4 weeks. Oxoid breeders diet was described as a reconstituted diet that allowed the ready incorporation of gum arabic into pellet form.

Feces were collected during the 24 h period before animals were killed. Following ad libitum overnight feeding, the animals were killed using a combination of diethyl ether anesthesia and cervical dislocation and contents from the stomach, small bowel, cecum, and distal colon were removed.

For rats fed gum arabic in the diet, a white flocculent precipitate typical of gum arabic was detected in contents from the stomach and small intestine, but not from the cecum, distal colon, or in the feces. The fact that precipitable gum arabic was detected along the gastrointestinal (GI) tract as far as the terminal ileum, but not in the cecum, suggests that the metabolism of gum arabic is mediated by bacteria in the cecum.

In animals in which the cecum was resected, precipitable gum arabic was detected along the length of the entire residual intestine. This observation suggests that in the absence of the bacterial mass resident in the cecum, there is no degradation of gum arabic. No precipitate typical of gum arabic was found in the GI tract of control rats that received the Oxoid breeders diet only (Ross et al. 1984b).

A total caloric intake slightly greater than that for starch has been reported for gum arabic in rabbits. Evidence of glycogenesis was also demonstrated in this study. Thus, it appears that rabbits are able to utilize gum arabic (FASEB 1973).

In a study involving guinea pigs, it was determined that gum arabic was highly digestible (90%) when administered in the diet at a concentration of 15% for 10 days (Informatics 1972).

Results of studies in which dogs and rabbits were injected intravenously with gum arabic indicated that gum arabic or some other product associated with it accumulated in the liver and remained in the tissues for several months. Nonlethal effects included serious disturbances in hemoglobin, white blood cells, and serum proteins (FASEB 1973).

Using many of the studies summarized above, the Select Committee on GRAS Substances determined in 1973 that gum arabic can be digested to simple sugars. However, it was also determined that conclusive evidence indicating that the intact gum arabic molecule is absorbed under normal conditions was lacking (FASEB 1973). It should also be noted that data on the fate of undigested gum arabic in male rats (Ross et al. 1984b) have been published since the FASEB report was issued. The results of this previously summarized study suggest that the bacterial mass resident in the cecum is responsible for the metabolism of gum arabic.

Hypotensive Activity

Acacia (Not Gum Arabic)

Sham et al. (1984) evaluated the hypotensive activity of *Acacia catechu* (aqueous extract of branches) using four groups of four anesthetized dogs (males and females; weights = 8 to 12 kg). The right femoral artery and vein were cannulated for blood pressure recordings and intravenous injection. After a 30-min equilibration period, *Acacia catechu* was injected (bolus injection) into dogs from each of the four groups. Doses ranged from <1 to ∼2 mg/kg. Changes in mean arterial blood pressure (MAP) were recognized as differences between the steady MAP before injection and the lowest MAP after injection.

The results were presented as a log dose-response curve. *Acacia catechu* induced dose-related hypotensive responses. At high doses, the hypotensive effect lasted approximately 30 min. Based on experimentation with various blocking agents, it was determined that this effect was not mediated through α- and β-adrenergic, cholinergic, or histaminergic receptors, or related to autonomic ganglion transmission.

The hypotensive activity of *Acacia catechu* (aqueous extract of branches) was also evaluated using four groups of five male Sprague-Dawley rats (weights between 170 and 250 g) according to the procedure in the preceding paragraph; however, in this experiment, the left carotid artery and jugular vein were cannulated.

Acacia catechu induced dose-related hypotensive responses in rats over the range of doses tested (1 to 2 mg/kg). It was also determined that the hypotensive responses were not mediated through α- and β-adrenergic, cholinergic, or histaminergic receptors, or related to autonomic ganglion transmission (Sham et al. 1984).

These same authors reported that, in an in vitro experiment, *Acacia catechu* induced a dose-dependent relaxation of helical strips of rat tail artery that had been preconstricted with the vasoconstrictors arginine vasopressin and methoxamine, respectively. In the presence of arginine vasopressin, *Acacia catechu* was tested at concentrations of 0.01, 0.03, and 0.1 mg/ml. *Acacia catechu* was tested at concentrations of 0.1, 0.3, and 1 mg/ml in the presence of methoxamine (Sham et al. 1984).

Hypocholesterolemic Activity

Acacia (Not Gum Arabic)

Chaudhari and Hatwalne (1973) determined the hypocholesterolemic activity of the dried water extract of *Acacia catechu*, also known as katha in India. They used three groups of 10 male albino rats (weights = 100 to 125 g). One group was fed stock diet thoroughly mixed with 1% cholesterol, and a second group was fed stock diet thoroughly mixed with 1% cholesterol plus 0.2% katha. The control group was fed stock diet only. The diets were fed ad libitum. Half of the animals in each group were killed after 6 weeks of feeding, and the remaining animals were killed after 12 weeks of feeding. The cholesterol content of the serum and liver was determined for each rat.

A progressive increase in serum and liver cholesterol content was observed in animals fed the stock diet supplemented with cholesterol for 6 months. In animals fed stock diet supplemented with cholesterol and katha for 6 months, the elevation of serum and liver cholesterol levels was significantly lower ($p =$ .001) when compared to rats fed stock diet supplemented with cholesterol.

However, at the end of 12 weeks, the increase in serum and liver cholesterol concentrations in rats fed stock diet supplemented with cholesterol and katha was elevated by approximately 50% when compared to rats fed stock diet supplemented with cholesterol only. It was also determined that there was substantially less deposition of lipids in the liver of katha-fed rats. It was concluded that katha had hypocholesterolemic activity in this study, and that it helped prevent fatty degeneration of the liver (Chaudhari and Hatwalne 1973).

Hypoglycemic Activity

Acacia (Not Gum Arabic)

Wassel et al. (1992) studied the hypoglycemic activity of ethanolic extracts of the pod, leaf, stem, old stem, and flower of *Acacia farnesiana L. Willd* using groups of 11 alloxanized diabetic albino rats (weights = 150 to 200 g). To prevent the development of fatal hypoglycemia during the first 12 h after alloxan administration, a 25% glucose solution (5 to 10 ml) was subcutaneously injected at 2 to 3 h intervals. Extract from each plant part (dose = 30 or 50 mg/kg in polysorbate 80) was administered orally to a group of 11 rats, and blood samples were taken at 2 h post administration. Blood samples were collected prior to treatment in order to estimate the normal blood glucose level of fasting rats.

The hypoglycemic activity of ethanolic extracts of *Acacia farnesiana* stem and pod was considerable following the administration of a 50 mg/kg dose. *Acacia farnesiana* stem and pod caused 21% and 36% reductions in the normal fasting blood sugar level, respectively (Wassel et al. 1992).

Effects on Smooth Muscle

Acacia (Not Gum Arabic)

Wassel et al. (1992) also studied the effect of ethanolic extracts of the pod, leaf, stem, old stem, and flower of *Acacia farnesiana L. Willd* on uterine motility. Rat uteri at various stages of the estrous cycle were suspended in 50-ml baths containing oxygenated Krebs solution; uteri were equilibrated in the solution for at least 90 min. Drugs were added to the water bath and were retained until the highest contraction was achieved.

Normal rhythmic contractions of the isolated uteri were first recorded using a T_2 isotonic transducer and two channel MD_2 oscillograph. Subsequently, the plant extracts (in polysorbate 80) were added to organ water baths at a dose of 50 or 75 mg/50 ml bath. The drug used to induce uterine contraction was then removed by washing the preparation with fresh Krebs solution.

Most of the *Acacia farnesiana* ethanolic extracts stimulated uterine muscular contraction during the estrous cycle and pregnancy. However, some of the extracts had a stimulatory effect on uterine contraction, followed by inhibition (i.e., leaf extract on non-estrus uteri and pod extract on pregnant uterus). The stem extract of *Acacia farnesiana* inhibited contraction of the pregnant uterus (Wassel et al. 1992).

Trivedi et al. (1986) evaluated the bronchodilator activity of *Acacia farnesiana* using the perfused, isolated guinea pig lung. The control guinea pig lung preparation was treated with saline. The unripe pods of *Acacia farnesiana* were collected and dried at room temperature. The glycosidal fraction of the ethyl alcohol extract of coarsely powdered Acacia pods was then isolated, and an aqueous solution of this fraction was tested.

Doses of 2, 5, and 10 μg of the aqueous solution increased outflow in the isolated lung perfusion preparation, indicating that the glycosidal fraction induced a smooth muscle relaxant effect. The same doses also increased outflow following histamine (10 μg)-induced contraction, and the bronchodilator effect was not blocked by propranolol (400 μg). These results suggested that the glycosidal fraction exerted a direct relaxant action on the bronchial muscles. The investigators noted that this effect is not mediated through β-adrenergic receptors.

The vasodilator activity of *Acacia farnesiana* was evaluated in vitro. The glycosidal fraction of the ethyl alcohol extract of coarsely powdered Acacia pods was isolated, and an aqueous solution of this fraction was tested. The hind limb of dogs was perfused through the femoral artery with oxygenated, defibrinated blood in Ringer's solution. Femoral venous outflow was recorded periodically. The control preparation was treated with normal saline.

The aqueous glycosidal fraction induced vasodilation at doses of 2, 5, and 10 μg (% increases in blood flow/min of 21.4, 20.86, and 24.3, respectively; $n = 5$). Vasodilation was not blocked following the addition of any of the following agents: chlorphenaramine maleate (20 μg), atropine (20 μg), or propranolol (400 μg). Study results indicated that the glycosidal fraction of *Acacia farnesiana* had a smooth muscle relaxant effect. The investigators noted that this effect was not mediated through cholinergic or H_1 receptors (Trivedi et al. 1986).

Anti-Inflammatory Activity

Acacia (Not Gum Arabic)

The anti-inflammatory activity of *Acacia farnesiana* was evaluated in vitro. The glycosidal fraction of the ethyl alcohol extract of coarsely powdered Acacia pods was isolated, and an aqueous solution of this fraction was tested. The effect of this fraction on chemically induced edema of the rat hind paw was evaluated according to the method of Winter et al. (1962). The glycosidal fraction inhibited carrageenin and formaldehyde induced inflammation of the rat hind paw in vivo (% inhibition of 38.2 and 26.26, respectively; $p < .001$, $n = 10$). It was concluded that this fraction has a promising anti-inflammatory effect (Trivedi et al. 1986).

Oxidative Phosphorylation

Gum Arabic

Bachmann et al. (1978) administered gum arabic twice daily to groups of four rats (weights = 100 to 110 g) at concentrations of 1%, 2%, and 10%, respectively, 5 days per week for 4 weeks. The test substance was suspended in distilled water and administered orally at a dose volume of 0.2 ml/100 g body weight; control rats were given equal volumes of distilled water. The actual doses of gum arabic administered were 2 × 20, 2 × 40, and 2 × 200 mg/kg/day. Groups of four rats were killed by cervical dislocation 16 h after administration of the last dose. Following maceration and homogenization, heart and liver mitochondria were isolated by differential centrifugation. Electron transfer reactions (oxygen consumption) and oxidative phosphorylation were measured polarographically. The hydroxylation of biphenyl was chosen as the assay system for measuring mixed function oxidases of hepatic cell endoplasmic reticulum.

Dose-dependent uncoupling of oxidative phosphorylation was the primary effect on cardiac and hepatic cell mitochondrial function. The damage to cardiac mitochondria progressed as dosing continued. However, hepatic cell mitochondrial function seemed to have gradually returned to normal during the fourth week of dosing.

At the highest administered dose (2 × 200 mg/kg/day) marked uncoupling of oxidative phosphorylation was observed in the heart and liver after 2 days of dosing. Partial recovery was reported for cardiac mitochondria after the first week of dosing; however, the same degree of uncoupling was noted up to the end of the experiment. Hepatic cell mitochondria were said to have recovered slowly as the experiment progressed. Gum arabic also caused a progressive inhibition of the biphenylhydroxylase system in the hepatic microsomal fraction (Bachmann et al. 1978).

Lutz et al. (1978) considered these results and investigated whether comparable biochemical effects of gum arabic (USP grade) could also be demonstrated in vivo. The measurement of maximal aminopyrine demethylation as expired CO_2 was deemed a suitable approach for this investigation, which was conducted using female rats of the ZUR SIV-Z strain (weights = 152 to 180 g). Oral dosing with 10% (w/v) gum arabic had no effect on the in vivo demethylation of 4-dimethyl[^{14}C]-aminoantipyrine (Lutz et al. 1978).

Antimicrobial Activity

Acacia (Not Gum Arabic)

The antimicrobial activity of ethanolic extracts of plant organs from *Acacia farnesiana* was evaluated. Extracts were made from the following plant parts: the pod, leaf, stem, old stem, and flower. Bacteria and yeast were cultured and filter paper disks were impregnated with 10 μl of each extract. Each disk (one extract per disk) was then dried and placed on the surface of the inoculated agar medium, and cultures were incubated for 48 h and observed for zones of inhibition. All plant extracts were inhibitory to *Bacillus subtilis* and *Staphylococcus aureus.* Additionally, most of the extracts were inhibitory to *Sarcina lutea*, *Pseudomonas aeruginosa*, and *Escherichia coli*. The plant extracts had no effect on *Mycobacterium phlei* or *Candida albicans* (Wassel et al. 1992).

ANIMAL TOXICOLOGY

Acute Oral Toxicity

Gum Arabic

In an acute oral toxicity study using rabbits (weights and strain not stated), an Acacia Gum LD_{50} of 80 g/kg was reported (Dangerous Properties of Industrial Materials Report 1981).

Acacia (Not Gum Arabic)

Letizia et al. (2000) conducted a study in which the acute oral toxicity of Acacia Farnesiana Extract (from flowers) was evaluated using ten rats (strain not stated). The test substance was administered at a dose of 5.0 g/kg, and animals were observed for 14 days. Necropsy was performed at the end of the observation period.

An LD_{50} of greater than 5.0 g/kg was reported. Signs observed in animals during the study included chromorhinorrhea in five or more animals and isolated instances of the following: tachypnea, chromodacryorrhea, ptosis, lethargy, piloerection, emaciation, ataxia, and respiratory noise. Necropsy findings for the only animal that died included abnormalities of the lungs, kidneys, liver, spleen, and gastrointestinal tract (Letizia et al. 2000).

The Societe Bertin (1987) reported an acute oral toxicity study in which Cire Essentielle Cassie (trade name for Acacia Farnesiana Flower Wax) was evaluated using groups of five rats (males and females) of the OFA Sprague-Dawley IOPS strain. Mean weights for male and female test animals were 219.60 g and 183.60 g, respectively. Control mean weights were 224.0 g (males) and 183.80 g (females). The animals were all approximately the same age (ages not stated). A single 10 ml/kg dose of the product was administered orally to each animal, and followed by a 14-day observation period. Control animals were dosed with corn oil (10 ml/kg). The animals were killed at the end of the observation period and necropsy performed.

Significant changes in general condition (weight changes included) or behavior between test and control animals were not observed. None of the animals died and no test substance-related organ lesions were observed. The test material was classified as innocuous at the dose administered (Societe Bertin 1987).

Biogir S.A. Conseil Recherche (1990a) also reported the acute oral toxicity of Cire Essentielle de fleurs de Mimosa (trade name for Acacia Dealbata Leaf Wax) in a suspension with paraffin oil using five male (178.3 $\pm$ 9.8 g) and five female (172.8 $\pm$ 5.9 g) rats of the OFA Sprague-Dawley strain (SPF). The animals were 2 months old. A single oral dose of 2 g/kg (10 ml/kg) of the product was administered to each animal by gavage, and dosing was followed by a 14-day observation period. Feeding resumed at 4 h post dosing. At the end of the observation period, the animals were killed and gross necropsy performed.

Weight gain was described as normal and no deaths were reported. Additionally, none of the animals had overt signs of central nervous system or neurovegetative system toxicity, and no lesions of organs examined were noted at necropsy. The minimal lethal dose was greater than 2 g/kg (Biogir S.A. Conseil Recherche 1990a).

Acute Dermal Toxicity

Acacia (Not Gum Arabic)

The acute dermal toxicity of Acacia Farnesiana Extract (from flowers) was evaluated using 10 rabbits (strain and weights not stated). A single dose of 5.0 g/kg was administered dermally to each animal, and observations were made over a period of 14 days. Gross necropsy was performed at the end of the observation period. Signs observed during the study were as follows: isolated instances of lethargy, diarrhea, ptosis, and nasal discharge (yellow). Gross observations at necropsy were normal for each animal. An LD_{50} of greater than 5.0 g/kg was reported (Letizia et al. 2000). Skin irritation reactions observed in this study are included in the section on Skin Irritation later in the report text.

Acute Intraperitoneal Toxicity

Gum Arabic

In a study using dogs (number and weights not stated), the intraperitoneal injection of 4.8 g/kg gum arabic did not induce toxicity. However, the same dose killed dehydrated dogs (highest no-effect level = 1.9 g/kg) (FASEB 1973).

Short-Term Oral Toxicity

Gum Arabic

Informatics Inc. (1972) reported a study in which diets containing Gum Arabic were fed to 133 guinea pigs. Except for one diet containing 20% gum arabic, all of the diets contained 15% gum arabic. The animals were fed for periods ranging from three to nine weeks. No toxic effects resulted from the administration of gum arabic.

Groups of rats (number and weights not stated) were fed 15% gum arabic in the diet for 62 days. A cathartic effect was noted. Weight gain, feed efficiency, hematological findings, and organ weights were normal (World Health Organization 1974).

Anderson et al. (1984) fed three groups of three male Albino Wistar rats (weights = 140 to 160 g) diets containing 1%, 4%, and 8% (w/w) gum arabic (Acacia Senegal Gum), respectively, daily for 28 days. A fourth group served as the negative control. At necropsy, hepatic and cardiac tissues were obtained for electron microscopy and microsomal P-450 assays.

No discernible ultrastructural differences were observed between the livers of test (all dietary groups) and control rats; particularly, the mitochondria were normal. Also, no discernible ultrastructural differences were found between the hearts of test (all dietary groups) and control rats. Particularly, both the appearance and concentration of the mitochondria and myofibrils were identical in this comparison. The results of assays of hepatic microsomal protein and cytochrome P-450 for each dietary group indicated that gum arabic did not cause inductive effects. The investigators noted that when induction by active agents (e.g., phenobarbitone) takes place, cytochrome P-450 values are increased by several-fold within a few days (Anderson et al. 1984).

Anderson et al. (1986) fed 10% (w/w) gum arabic (Acacia Senegal Gum) daily for 45 days to Wistar albino rats (99 to 120 g). The number of rats in the study was not stated. The rats were then killed by cervical dislocation while under ether anesthesia. Portions of the jejunum, ileum, and cecum were excised and the ultrastructure of each was evaluated using transmission electron microscopy.

No abnormalities in organelles were observed within cells of the jejunum, ileum, or cecum of rats fed gum arabic. Additionally, neither inclusions nor other pathological changes were detected. It was concluded that no significant ultrastructural differences occurred between experimental and control rats (Anderson et al. 1986).

Cook et al. (1992) evaluated the oral toxicity of gum arabic (Acacia species not stated) using 3-week-old Sprague-Dawley rats (16 males, 16 females). Three days before dosing, mean body weights were 122 g and 125 g for males and females, respectively. The animals were fed gum arabic (dose not stated) daily for 28 days and then killed by exsanguination. Blood samples were obtained for hematological examination and serum analysis the day before animals were killed. Microscopic examination of most organs was performed, which included examination of any tissues that appeared abnormal.

No treatment-related behavioral effects were noted. All values for serum chemistry parameters were within the normal limits for laboratory rats. Mean red blood cell volume values were said to have been within the normal range for Sprague-Dawley rats. No toxicologically significant lesions were noted at microscopic examination (Cook et al. 1992).

Short-Term Intravenous Toxicity

Gum Arabic

Acacia (Gum Arabic) was administered intravenously to three dogs (weights not stated) over a period of 76 days. The number of intravenous injections ranged from 32 to 35 over this period, and the range for the total cumulative dose was 15.7 to 47.7 g/kg. An enlarged liver was observed in the dog that received the greatest cumulative dose; death occurred four months after the last injection. The cause of death was not stated. The remaining two dogs remained in good condition. The results of biopsies performed on the two animals indicated that Acacia was present in the liver 26 months after the last injection (World Health Organization 1974).

In another study, gum arabic was administered intravenously to dogs (number and weights not stated) over a period ranging from 1 to 84 days. Doses ranged from 1 to 2 g/kg. Enlarged livers and swollen kidneys were the most characteristic changes. Similar doses were fatal when administered to two rabbits (weights not stated) (FASEB 1973).

Subchronic Oral Toxicity

Gum Arabic

Anderson et al. (1982) evaluated the subchronic oral toxicity of gum arabic (Acacia Senegal Gum) in two experiments using albino Wistar rats (24 to 28 days old). Body weights prior to initiation of the study were not included.

In the first experiment, groups of 15 male rats were fed gum arabic at concentrations of 0.91% (dietary level = 0.53 g/kg/day), 2.0% (1.08 g/kg/day), 4.3% (2.55 g/kg/day), and 8.6% (5.22 g/kg/day), respectively, for 13 weeks. Groups of 15 female rats were fed concentrations of 0.75% (0.5 g/kg/day), 1.7% (1.05 g/ kg/day), 3.7% (2.6 g/kg/day), and 7.5% (5.31/g/kg/day), respectively. Fifteen males and 15 females served as controls.

In the second experiment, 15 male rats were fed gum arabic at an average concentration of 18.6% (14 g/kg/day) for 13 weeks. Fifteen female rats were fed an average concentration of 18.1% (13.8 g/kg/day). The two control groups consisted of 15 males and 15 females, respectively. Urine and blood samples were obtained during the study. The animals were killed under anesthesia by cervical dislocation at the end of the treatment period and prepared for necropsy.

The results for the two experiments included the reported deaths of two control female rats. Growth rates were not reduced for male or female rats at dietary doses up to 5 g/kg/day (~8.5% gum arabic in diet). At a concentration of approximately 18% in the diet (14 g/kg/day), male rats had a reduced growth rate and smaller final body weight ($p < .01$). The average weight gain for male rats was 78% of that of controls.

Following the ingestion of gum arabic, 5 g/kg/day, by male rats, kidney weights (absolute and relative to body weight) were reduced ($p < .05$). At the highest dietary doses tested (∼18%, 14 g/kg/day), kidney weights for male and female rats were significantly reduced ($p < .01$). Liver weight was reduced in a dose-dependent manner in male rats; the difference between experimental and control groups was not significant at doses of gum arabic less than 5 g/kg/day. No significant differences were observed in urine volume or composition between control and test groups at any of the dietary concentrations of gum arabic tested.

Similarly, no significant hematological changes were observed between test and control groups. At microscopic examination, no alterations were found that were attributable to the ingestion of gum arabic. The only treatment-related alteration noted at necropsy was cecal enlargement in rats of the highest-dose groups (Anderson et al. 1982).

In another study, Anderson et al. (1984) fed four groups of five male albino Wistar rats (weights = 40 to 60 g) diets containing 0.5%, 1.5%, 2.5%, and 3.5% (w/w) gum arabic (Acacia Senegal Gum), respectively, daily for 91 days. A fifth group served as the negative control. At the end of the feeding period, the animals were killed by cervical dislocation for necropsy. Samples of liver and heart from each treatment group were obtained for transmission electron microscopy. Livers from the remaining rats (two per group) were used for assays of microsomal protein and cytochrome P-450.

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

Skin Irritation

Acacia (Not Gum Arabic)

In an acute dermal toxicity study, Acacia Farnesiana Extract (from flowers) was administered dermally (single dose of 5.0 g/kg) to 10 rabbits, after which animals were observed for 14 days. On day 1, moderate erythema and moderate edema were observed in all ten rabbits (Letizia et al. 2000).

Biogir S.A. Conseil Recherche (1990b) evaluated the skin irritation potential of Cire Essentielle de fleurs de Mimosa (trade name for Acacia Dealbata Leaf Wax) using six New Zealand albino rabbits. The undiluted product (volume = 0.5 ml on an occlusive patch) was applied to scarified skin (clipped free of hair) of the right flank of each animal. The left flank (nonscarified skin) of each animal served as the control. Each patch was secured with a hypoallergenic, microporous adhesive strip and an elastic band (fixed with adhesive tape) that was wrapped around the trunk. Patches were removed after 24 h of contact.

At 24 h and 72 h post application, reactions were scored according to the following grading scales: 0 (no erythema) to 4 (severe erythema [crimson red] with or without eschar [deep injuries] and lesions showing a serious cutaneous reaction such as a burn, a necrosis) and 0 (no edema) to 4 (severe edema [more than 1 mm thick and extending beyond the area of exposure] showing a serious cutaneous reaction such as a burn). Scores for erythema and edema (intact and scarified skin) were determined at 24 and 72 h post application. The scores (intact and scarified skin) obtained were added together and divided by 24 to calculate the primary cutaneous irritation index.

Cire Essentielle de fleurs de Mimosa (Acacia Dealbata Leaf Wax) was classified as a nonirritant (primary irritation index = 0.5) in New Zealand albino rabbits (Biogir S.A. Conseil Recherche 1990b).

Bertin Laboratories (1987) reported on a study in which the skin irritation potential of Cire Essentielle Cassie (Acacia Farnesiana Flower Wax) was evaluated using six New Zealand rabbits. The test substance (in pure form, 0.5 ml under occlusive pad) was applied to intact and scarified skin of the right and left flank, respectively, that had been clipped free of hair. A stretch bandage was then wound around the torso of each animal and secured with adhesive tape. Patches were removed at 24 h post application. Reactions were scored at 24 and 72 h post application according to the following grading scales: 0 (no erythema) to 4 (serious erythema [purple red] with slight scarring [deep lesions] and 0 (no edema) to 4 (serious edema [over 1 mm thick] with a surface area greater than 1 mm^2).

At 24 h, reactions at intact sites were described as somewhat pronounced erythema (2 rabbits [slight erythema, score = 1]; 1 rabbit [highly visible erythema, score = 2]). Very slight edema (score = 1, intact site) was also noted in the rabbit with highly visible erythema. Identical results were reported for scarified skin sites. At 72 h, reactions were observed in one rabbit; slight erythema and very slight edema were observed at intact and scarified sites. Cire Essentielle Cassie (Acacia Farnesiana Flower Wax) was classified as a slight skin irritant (primary cutaneous irritation index = 0.6) (Bertin Laboratories 1987).

Phototoxicity

Acacia (Not Gum Arabic)

The phototoxicity of a 20% solution of Acacia Farnesiana Extract (from flowers) in methanol was evaluated using six SKH:hairless mice. The test substance was applied to a 5-cm^2 area on the back of each animal. At 30 min post application, test sites were irradiated with UV light for 1 h. The light source consisted of a bank of six fluorescent, black light lamps positioned at a distance of 35 cm, or an Atlas xenon lamp (model Rm 60 or 65 [wavelength: 280 to 320 nm] with a Schott WG320 filter) positioned at a distance of 1 m. Reactions were scored at 4, 24, 48, 72, and 96 h post exposure. No phototoxic effects were observed (Letizia et al. 2000).

Immunological Responses

Studies on immunological responses to gum arabic and Acacia solution/extract are summarized in Table 9.

Acacia (Not Gum Arabic)

Maytum and Magath (1932) reported a series of three experiments that evaluated the allergenicity of an Acacia solution (exact composition not stated). In the first experiment, six rabbits (12 weeks old) were injected intravenously with 50 cc Acacia, and this dose was repeated 5, 12, and 17 days later. At 4 weeks after the last injection, each rabbit was injected intravenously with 2 cc of Acacia.

The rabbits appeared normal during a 1-h observation period following this injection. On the same day, one of the rabbits was injected intravenously with 2 cc of a 50% egg white solution to determine whether exposure to a foreign protein would result in greater sensitivity to Acacia. Acacia (2 cc) was injected intravenously 3 weeks later, and then 3 weeks after this injection at a dose of 15 cc. No signs of anaphylaxis were observed in this animal (Maytum and Magath 1932).

In the second experiment, eight guinea pigs (weights = 300 g) were injected intraperitoneally with a dose of 10 cc, and this dose was repeated 5, 12, and 17 days later. At four weeks after the last dose, two of the animals were injected intravenously with 0.5 cc Acacia.

Typical anaphylactic signs (sneezing and coughing, scratching the nose, and dyspnea) were noted in both guinea pigs after approximately 30 s. The two animals died approximately 3 min after signs were first noted. Two other guinea pigs were injected intracardially with Acacia solution (0.5 cc; exact composition not stated), after which both had milder signs of anaphylaxis. One animal recovered, and the other died after 1 h. The remaining four guinea pigs each received an intraperitoneal injection of Acacia solution (0.5 cc). Mild reactions were noted in two of the animals, and no signs were reported for the remaining two.

A follow-up third experiment was performed to determine whether the guinea pig deaths reported were due to the intravenous method of test substance administration in the second experiment. Four guinea pigs were injected intravenously with 0.5 cc Acacia solution (exact composition not stated), and no deleterious effects were noted. Acacia solution (10 cc) was administered intraperitoneally to eight guinea pigs; four of the animals died within five days after injection.

Seven days later, intraperitoneal injections of Acacia solution (10 cc) were given to the four remaining guinea pigs (from second experiment) that were injected intraperitoneally, the four guinea pigs that were injected intravenously in the first experiment, and four new guinea pigs. Of the four new guinea pigs, two died from peritonitis within 4 days.

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

In total, these authors reported on the results of studies involving a total of 19 guinea pigs (8 guinea pigs from preceding experiment included) that include sensitization induced by Acacia solution (administered parenterally; exact composition not stated) and no anaphylactic signs developed in seven of the animals.

Mild and moderate anaphylactic signs developed in four and three guinea pigs, respectively, and severe signs were noted in two guinea pigs. Three of the 19 guinea pigs died. In addressing the results from the preceding experiments, the investigators noted that anaphylactic sensitivity to Acacia can develop under certain unusual conditions. It was also stated that no danger was associated with an initial dose of Acacia if the solution was properly prepared; however, subsequent doses administered after at least 3 weeks should be given cautiously because of the possibility of anaphylactic reactions (Maytum and Magath 1932).

Aronson and McMaster (1972) sensitized 12 guinea pigs (strain not stated; weights ≈ 300 g) via single intra-abdominal injections of 600 mg Acacia (6% solution, 10 ml; composition of solution and Acacia species not stated). The animals were challenged 1 month later with an intravenous injection of 60 mg of the sensitizing sample or other samples of Acacia.

The nonnecrotizing toxicity of Acacia extract was evaluated using germ-free and conventional guinea pigs of the Hartley strain. The ages of the germ-free animals tested were as follows: group A (12 animals, 8 days old), group B (9 animals, 3 weeks old), and group C (6 animals, 12 weeks old). The test substance (40 mg/ml) was suspended in phosphate buffer (pH 7.4, 0.1 M) and applied topically to the cornea of the right eye; phosphate buffer was applied to the cornea of the left eye. For both substances, one drop was applied every half hour for a total of seven applications.

The following three groups of conventional guinea pigs were also treated according to the same procedure: group 1 (six animals, 8 days old), group 2 (seven animals, 12 weeks old), and group 3 (two animals, 7 months old). All animals were killed 30 min after application of the last drop. Additionally, phosphate buffer was instilled into both eyes of two animals (killed when 8 days old), and the same was true for two other animals (killed when 3 weeks old). The eyes were enucleated immediately after all animals were killed. The animals were bled prior to killing, and serum samples were subsequently obtained for determination of antibody or y-globulin. At microscopic examination, a severe inflammatory response was observed in both germ-free and conventional 8-day-old guinea pigs.

TABLE 9
Immunological responses

Test substance	Animals tested	Test procedure	Results	Reference
Acacia solution	6 rabbits (12 weeks old)	Four i.v. injections (50 cc) on days 0, 5, 12, and 17, followed by single i.v. injection (2 cc) 4 weeks after fourth injection	No signs of anaphylaxis	Maytum and Magath 1932
Acacia solution	8 guinea pigs (weights = 300 g)	Four i.p. injections (10 cc) on days 0, 5, 12, and 17 followed by single i.v. injection (0.5 cc) 4 weeks after fourth injection	Anaphylactic signs (sneezing, coughing, dyspnea) in 8 animals; 2 deaths. Milder signs noted in 2 surviving animals injected intracardially (0.5 cc); 1 died. Mild signs also in 2 of remaining 4 survivors injected intraperitoneally (0.5 cc). In a follow-up experiment involving guinea pigs, it was concluded that Acacia was capable of inducing peritonitis (followed by death) regardless of the route of administration, i.p. or i.v.	Maytum and Magath 1932
Acacia solution	19 guinea pigs (8 guinea pigs in preceding study included)	Parenteral administration	No anaphylactic signs (10 animals); mild and fairly severe anaphylactic signs in 4 and 3 animals, respectively; extremely severe signs in 2 animals; 3 of 19 died	Maytum and Magath 1932
Anti-Gum Acacia rabbit serum	5 guinea pigs (weights = 300 to 450 g)	Passive sensitization with 2 ml of serum (i.p. injection), followed by i.v. dose of a homologous gum (1 mg)	3 animals died at 2 to 3 min post injection. The remaining 2 recovered from anaphylactic shock slowly	Partridge and Morgan 1942
7% Gum Acacia solution	Two groups of 10 guinea pigs (weights = 600 to 1000 g)	Injected subcutaneously (5 ml) repeatedly over 7-week period. After 2 weeks of dosing, animals injected with 1 ml *Brucella abortus* vaccine	No deleterious effects on antibody production resulted, as judged by the development of agglutinative and complement-fixing activity in the serum to *Brucella abortus*	Rice 1954a
7% Gum Acacia solution	4 rabbits (weight range = 1800 to 2650 g)	Injected subcutaneously (10 ml) repeatedly over 4-week period. Injected with *Brucella abortus* vaccine 4 days (2 ml) and 8 days (3 ml) later	No deleterious effects on antibody production resulted, as judged by the development of agglutinative and complement-fixing activity in the serum to *Brucella abortus*	Rice 1954a

(Continued on next page)

TABLE 9
Immunological responses *(Continued)*

Test substance	Animals tested	Test procedure	Results	Reference
7% Gum Acacia solution	Two groups of 10 guinea pigs	Group1: Injected subcutaneously (5 ml) repeatedly over 16-day period. Actively sensitized after seven doses and challenged in 3 weeks Group 2: Received 11 subcutaneous injections. Passively sensitized and challenged 48 h later	Group 1: One animal with signs of asphyxia; 8 animals with shock signs; 2 died Group 2: Typical respiratory signs developed; no deaths Both groups: No significant decline in serum-complement activity	Rice, 1954b
7% Gum Acacia solution	Two groups of 10 guinea pigs	Group1: Injected subcutaneously (5 ml) repeatedly over 16-day period. Actively sensitized after seven doses and challenged in 3 weeks Group 2: Received 11 subcutaneous injections. Passively sensitized and challenged 48 h later	Group 1: One animal with signs of asphyxia; 8 animals with shock signs; 2 died Group 2: Typical respiratory signs developed; no deaths Both groups: No significant decline in serum-complement activity	Rice 1954b
6% Acacia solution	12 guinea pigs (weights = 300 g)	Twelve animals sensitized via single intra-abdominal injections of 600 mg Acacia (6% solution, 10 ml). Challenged 1 month later with i.v. injection of solution or other samples of Acacia. Two additional guinea pigs tested subsequently with Acacia from different lot	Twelve animals with anaphylactic shock; 10 died. Two additional guinea pigs sensitized by intra-abdominal injection of 160 mg Acacia with Freund's adjuvant (2 ml of emulsion containing two parts 20% Acacia), followed by i.v. challenge with 60 mg Acacia 1 month later, died of anaphylactic shock	Silvette et al. 1955
Three grades of Gum Arabic (dissolved in 0.15 M NaCl at concentration of 4 mg/ml). One of the grades was derived from food grade gum arabic	Groups of 6 to 8 female CBA mice (6 weeks old)	Mice immunized by injection of the antigen (0.1 mg in 0.05 ml Freund's adjuvant) into footpad. Delayed-type hypersensitivity measured 21 days after primary immunization	Compared to controls, no significant increase in footpad thickness. Antigen-specific hypersensitivity reaction noted for all three grades of gum arabic	Strobel et al. 1982

Gum Arabic (dissolved in 0.15 M saline at concentration of 400 mg/ml)	Two groups of 8 female BDF1 [(C57BL/6] × DBA/2 F_1] mice (6 to 8 weeks old)	Initially dosed with Gum Arabic (80 mg) by intragastric administration. Mice then immunized by injection of 100 μg gum arabic in saline and complete Freund's adjuvant into hindpaw. Delayed hypersensitivity measured at 3 weeks post immunization	Compared to controls, footpad swelling significantly suppressed. Systemic immunological hyporesponsiveness (oral tolerance) developed in mice fed gum arabic	Strobel and Ferguson 1986
Five different samples of Gum Arabic (*Acacia senegal*)	5 groups of 6 to 8 male [(C57BL/6J] × DBA/2 F_1] (BDF_1) mice	Footpad swelling test. Unimmunized male mice injected intradermally with each sample	All but one sample induced footpad swelling at 24 h. Footpad swelling said to have been indicative of nonspecific irritant effect	Strobel et al. 1986
Five different samples of Gum Arabic (*Acacia senegal*), each emulsified in Freund's complete djuvant	5 groups of 30 to 40 [(C57BL/6J] × DBA/2 F_1] mice	Footpad swelling test. Initially, mice immunized with each sample (200 μg per sample) in left hind footpad. Presence of delayed-type hypersensitivity measured	All samples found to be immunogenic. Intradermal challenge after immunization caused significant increase in footpad thickness at 24 h	Strobel et al. 1986
Five different samples of gum arabic (*Acacia senegal*), each emulsified in Freund's complete adjuvant	5 groups of 30 to 40 [(C57BL/6J] × DBA/2 F_1] mice	Test for cross-reactivity. Blood samples obtained from mice in preceding experiment at 3 weeks post immunization. Antibodies assayed using enzyme-linked immunosorbent assay (ELISA)	Except for one sample, assay results indicated that antigens were shared between the samples tested	Strobel et al. 1986
Acacia Extract	Germ-free and conventional guinea pigs of Hartley strain	Acacia Extract (40 mg/ml) applied topically to the right eye	Microscopic examination results: Severe inflammatory response observed in germ-free and conventional guinea pigs (14 animals total, 8 days old). Minimal inflammatory response in germ-free and conventional guinea pigs (13 animals total, 12 weeks old). Inflammatory response most severe in conjunctiva	Aronson and McMaster 1972

The inflammatory response was described as minimal in 12-week-old germ-free and conventional guinea pigs. In the 7-month-old conventional animals, the responses were much more severe than that noted for 12-week-old germ-free animals. This comparison was made because 7-month-old germ-free animals were not available.

The inflammatory response to Acacia was most severe in the conjunctiva and the subconjunctival tissues were relatively free of inflammatory changes. Swelling of superficial epithelial cells of the central cornea and necrosis of a few of these cells were also observed. The severity of inflammatory responses was correlated with serum y-globulin concentrations. The extent of the inflammation induced by Acacia paralleled y-globulin concentrations in germ-free guinea pigs more closely than in conventional guinea pigs (Aronson and McMaster 1972).

Gum Arabic

Five guinea pigs (weights = 300 to 450 g) were passively sensitized with 2 ml of anti-Gum Acacia rabbit serum via intraperitoneal injection. At 24 to 36 h post injection, an intravenous dose of a homologous gum (1 mg) was administered to each animal, and the animals were observed for signs of anaphylaxis. Three guinea pigs died 2 to 3 min after intravenous administration, and the remaining two slowly recovered from shock during the following 2 to 3 h (Partridge and Morgan 1942).

Rice (1954a) evaluated the effect of Gum Acacia (species not stated) on complement and antibody production using two groups of 10 guinea pigs (strain not stated; weights = 600 to 1000 g). The animals were injected subcutaneously with gum arabic (7% solution, 5 ml) on alternate days prior to and during immunization; gum arabic was injected repeatedly over a period of seven weeks. After 2 weeks of dosing, the animals were bled and injected intraperitoneally with 1 ml of *Brucella abortus* vaccine. Three additional injections of this vaccine were made 4 days (2-ml injection), 8 days (3 ml), and 21 days (3 ml) later.

The guinea pigs were bled again one week after the third and fourth doses of vaccine, and all sera were titrated for hemolytic complement and for agglutinative and complement-fixing activity with *Brucella abortus* antigens. Surviving animals were retested for 6 weeks, bled again, injected with a fifth dose of vaccine, and bled for a fourth time 7 days later. Twenty guinea pigs of comparable weight were included in each of the control groups (immunized and non-immunized).

A sharp decline in complement titers was noted in both groups of guinea pigs injected with Gum Acacia. Following seven injections, only 2 of 18 surviving guinea pigs had complement titers over 1000 units per ml (minimum titer = 455). After 14 injections, one of the remaining animals had a titer that approached normal (minimum titer = 385). During the ensuing period, a rise in complement titer to over 1000 units per ml was noted for five guinea pigs and complement titers below 500 units were noted for eight guinea pigs; the reason for these changes in titer was undetermined. In addition to the reductions in complement titer noted in the two groups, antibody and total serum protein production were also reduced. It was determined that no deleterious effects on antibody production resulted, as judged by the development of agglutinative and complement-fixing activity in the sera to the bacterial antigen *Brucella abortus* (Rice 1954a).

Rice (1954a) also evaluated the effect of Gum Acacia (species not stated) on complement and antibody production using four rabbits (weight range = 1800 to 2650 g). This experiment is from the study summarized in the preceding paragraph. The rabbits were injected subcutaneously with a 7% solution of Gum Acacia (10 ml) every other day for 4 weeks. All rabbits were bled on the 15th day and immunized with 1 ml *Brucella abortus* vaccine. The vaccine was also injected 4 and 8 days later in 2-ml and 3-ml volumes, respectively. The rabbits were bled again seven days after the third dose of vaccine. Untreated rabbits (immunized) and nonimmunized rabbits served as controls.

In contrast to the effects noted in guinea pigs in the preceding study, Gum Acacia did not appreciably lower complement activity. The authors concluded that no deleterious effects on antibody production resulted, as judged by the development of agglutination and complement-fixing activity in the sera to the bacterial antigen *Brucella abortus* (Rice 1954a).

In another study, Rice (1954b) evaluated complement titers in guinea pigs (strain and weights not stated) that were either actively or passively sensitized to a 7% solution of Gum Acacia. Ten guinea pigs were injected subcutaneously with 16 doses (5 ml per dose) of a 7% Gum Acacia (species not stated) solution over a period of 16 days. The animals were actively sensitized after 7 doses, and the nine survivors were bled, challenged, and rebled in 3 weeks.

Signs of asphyxia were reported for one of the nine survivors; this animal survived for more than 3 h. The other guinea pigs became excited shortly after challenge, running around wildly and squealing (shock signs); two eventually died. An additional 10 guinea pigs that had received 11 injections of Gum Acacia solution were passively sensitized, bled, and challenged 48 h later. Typical respiratory signs developed; none of the animals died. No significant decline in serum-complement activity was detected in animals challenged shortly after passive sensitization or in actively sensitized Gum Acacia-treated guinea pigs; however, a decline in this activity was noted. Additionally, in both sensitized groups, initial excitement followed by fatigue and weakness were the most striking clinical signs (Rice 1954b).

Silvette et al. (1955) reported that anaphylactic shock resulted in each of the 12 guinea pigs sensitized via intra-abdominal injection of 160 mg Acacia with Freund's complete adjuvant (FCA) (2 ml of emulsion containing two parts 20% Acacia). Ten guinea pigs died. Two additional guinea pigs were sensitized via intra-abdominal injection of 160 mg Acacia with FCA (2 ml of emulsion containing two parts 20% Acacia). This Acacia sample was from another lot. The animals were challenged intravenously with 60 mg Acacia 1 month later. Typical anaphylactic death was reported for both guinea pigs.

The results of this experiment as well as additional experiments (rabbits and guinea pigs) in this study collectively indicated that four different lots of Gum Acacia were equally effective as immunizing, sensitizing, and anaphylactogenic and desensitizing antigens, based on the results of cross-precipitin tests and cross-anaphylaxis experiments (Silvette et al. 1955).

Antibodies directed against gum arabic (species not stated) have been isolated using affinity chromatography on AH-Sepharose 4B containing gum arabic ligands. These antibodies were induced in rabbits immunized with gum arabic in FCA. It was determined that the antibodies were anti-carbohydrate antibodies with specificity for certain carbohydrate units of the gum arabic. The results of chemical modification and inhibition experiments indicated that 4-α-L-arabinofuranosyl-D-glucuronic acid units of the polysaccharide were the major immunodeterminant groups (Pazur et al. 1986).

Blood group antigens have been demonstrated in gum arabic (species not stated). The following substances were identified using an agglutinin inhibition test of mild hydrolyzed gum arabic: B, C (of ABO blood group system) and H substances (of H blood group system) and Le^a (Lewis^a antigen, in Lewis blood group system). The results of a revised latex agglutination technique indicated the presence of P and S (of MN blood group system) as well as the substances mentioned in the preceding statement. Elution processes, using sensitized and agglutinated latex or kaolin particles, resulted in the identification of B, H, and Le^a substances in gum arabic; the elution of anti-P and anti-S did not occur (Matsuzawa 1968).

Narita (1985) reported the isolation of high-titer anti-Gum Arabic sera obtained from rabbits injected with gum arabic (species not stated). The antisera had cross-reactivity with the Lewis^a antigen (Le^a), as measured by both a single diffusion tube test and the Ouchterlony test (Narita 1985).

Strobel et al. (1982) evaluated the allergenicity of three grades of gum arabic using female CBA mice (6 weeks old; six to eight mice per group). The grades of gum arabic tested were as follows: (1) processed gum arabic recovered by spray-drying from a solution of commercial food grade gum arabic after filtration to remove sand, etc., and after heat treatment to effect pasteurization; (2) finely powdered natural gum arabic of poor commercial quality giving solutions of a dark red-brown color; (3) finely powdered natural gum arabic of very high quality, giving essentially colorless solutions.

The gum exudates were dissolved in 0.15 M NaCl at a concentration of 4 mg/ml by incubation at 37°C for 16 h. The resulting solution was sterilized by irradiation. The mice were immunized by injection of the antigen (0.1 mg in 0.05 ml of FCA) into the left hind footpad. At 21 days after primary immunization, delayed-type hypersensitivity was measured using a skin test. In this test, the antigen (0.1 mg dissolved in 0.15 M saline in volume of 0.05 ml) was injected intradermally into the plantar side of the right footpad of anesthetized mice. Using a micro caliper, footpad thickness was measured in triplicate immediately before intradermal injection and 24 h later. For controls, footpad swelling was measured before and after antigen injection into the footpad of nonimmunized mice, and before and after saline injection into the footpad of immunized mice. All mice were killed one week after the skin tests. The animals were bled and serum separated and decomplemented.

The intradermal injection of antigen into nonimmunized, control mice (four mice per antigen) did not induce significant footpad swelling at 24 h. Similarly, the intradermal injection of saline into immunized control mice did not cause a significant increase in footpad thickness. However, compared to the control, significant positive responses were noted in mice of the test groups ($p < .01$), indicating an antigen-specific hypersensitivity reaction for all three gum arabic specimens that were tested. A comparison of results for the three grades of gum arabic indicated that footpad swelling in mice immunized and tested with the dark, red-brown grade was significantly greater ($p < .005$) when compared to the colorless grade (Strobel et al. 1982).

Strobel and Ferguson (1986) studied the immunological activity of gum arabic (species not stated) using two groups of eight female BDF1 [(C57BL/6] × DBA/2)F_1] mice (6 to 8 weeks old). A finely powdered sample of gum arabic was dissolved in 0.15 M saline at a concentration of 400 mg/ml. Each of eight mice was then dosed with gum arabic (80 mg) by intragastric administration. Control mice were dosed with saline. At 7 days post dosing, the mice were immunized by injecting a saline solution of 100 μg gum arabic emulsified in an equal volume of FCA (total volume injected = 0.05 ml) into the left hind footpad.

Control mice were immunized with 0.15 M saline in FCA. Prior to and 3 weeks after immunization all mice were bled and decomplemented sera were tested for anti-Gum Arabic antibodies by a micro-ELISA (enzyme-linked immunosorbent assay) technique.

Delayed-type hypersensitivity was also measured (skin test) at 3 weeks post immunization. The mice were anesthetized and 0.1 mg gum arabic (in volume of 0.05 ml) was injected intradermally into the right footpad. Footpad thickness was measured in triplicate immediately before intradermal injection and 24 h later. As controls, footpad swelling was measured before and after gum arabic was injected into the footpad of saline/adjuvant-immunized animals, as well as before and after saline was injected into the footpad of mice immunized with gum arabic.

Footpad swelling was negligible in both control groups. Antibodies were not detected in the serum of mice that were bled before systemic immunization. Serum antibodies were identified in five of eight control (saline prefed) mice after systemic immunization. However, antibodies were not detected in the serum of mice that were prefed with gum arabic. Regarding delayed-type hypersensitivity, a similar pattern was noted. Positive skin tests were reported for all saline-prefed mice. However, footpad swelling in mice prefed with gum arabic was significantly suppressed. Test results indicated that systemic immunological hyporesponsiveness (oral tolerance) developed in mice that were fed gum arabic (Strobel and Ferguson 1986).

Strobel (1986) evaluated the immunogenicity, cross-reactivity, and nonspecific irritant properties of gum arabic (Acacia Senegal Gum) using male mice (6 to 8 weeks old) of the [(C57BL/6J × DBA/2F_1] (BDF_1) strain. Nonspecific irritant properties were assessed in the foot pad swelling test using control groups of nonimmunized mice. Immunogenicity was evaluated in an in vivo footpad swelling test, and cross-reactivity was assessed by secondary antibody response.

The following gum arabic samples (identified as samples A, B, C, D, and E) were tested in each experiment: (1) Sample A (sodium arabate) resulted from the neutralization of sample C with sodium hydroxide. (2) Sample B resulted from three successive precipitations of sample C from aqueous solution with acidified ethanol. (3) Sample C, gum arabic, was a water-soluble polysaccharide containing rhamnose, arabinose, glucuronic acid, and galactose. (4) Sample D was defined as powdered food grade natural gum arabic. (5) Sample E was obtained by exhaustive ethanolic extraction of sample D. In the nonspecific footpad swelling test, five groups (six to eight mice per group) of nonimmunized male mice were injected intradermally with the five samples, respectively.

Sample A did not induce significant swelling at 24 h; however, samples B, C, and D increased, but only slightly, nonspecific swelling ($p < .05$). Sample E induced the greatest extent of footpad swelling. These results (footpad swelling) were indicative of a nonspecific irritant effect.

In a second experiment, five groups (30 to 40 mice per group) of mice were immunized with the five gum arabic samples (200 μg per sample), respectively, in the left hind footpad. Each gum arabic sample was emulsified in FCA prior to immunization. Control mice (30 to 40 mice) were immunized with saline in FCA. At 21 days post immunization, the presence of delayed-type hypersensitivity (specific cell mediated immunity) was measured in the footpad swelling skin test.

All gum arabic samples were immunogenic in this test. In each case, intradermal challenge after immunization caused a significant increase in footpad thickness at 24 h. In the test for cross-reactivity, blood samples were obtained from mice that had been immunized and tested (footpad swelling test) 3 weeks after immunization. Antibodies were assayed by an ELISA.

Assay results indicated that antigens were shared between all of the samples, except for sample E. Mice immunized with sample A had significant reactions when tested with samples A, B, C, and D. The greatest nonspecific swelling was produced by samples B and C (Strobel et al. 1986).

GENOTOXICITY

Gum Arabic

Both in vitro and in vivo studies on the mutagenicity of gum arabic described as gum arabic, Acacia, or Gum Acacia are summarized in Table 10. Although a few positive results are described, most studies were negative for genotoxicity.

UV Damage Repair

Acacia (Not Gum Arabic)

Jain et al. (1987) evaluated the effect of *Acacia arabica* on UV-induced damage in the WP-2 strain of *Escherichia coli*. Cultures were irradiated with UV light (1.5 J/m^2/s) for 15 s, with intermittent stirring. The bark of *Acacia arabica* was extracted with methanol and the extract was added to cultures at a concentration of 5 mg/plate. The revertants and viable cells were counted after incubation for two days at a temperature of 37°C.

Compared to control cultures exposed to UV light (mean number of revertants per plate = 216), the mutagenic activity of UV light was reduced in cultures dosed with *Acacia arabica* extract. The mean number of revertants per plate in test cultures was 34. The survival for control and test cultures was 100% and 70.6%, respectively. The investigators stated that the decrease in UV-induced mutagenicity in the presence of Acacia could have been due to some enzymatic action that reverted the formation of pyrimidine dimers (Jain et al. 1987).

Effect on Genotoxicity of Other Agents

Gum Arabic

The effect of 3% gum arabic (solvent) on the mutagenicity of 4-nitroquinoline-*N*-oxide was evaluated using results from the bone marrow micronucleus assay. Based on an analysis of time-response and dose-response data on 4-nitro-quinoline-*N*-oxide, it was determined that the mutagenicity of this chemical was six times greater in gum arabic when compared to test results for the chemical in DMSO. When the mutagenicity of other chemicals, such as mitomycin C, was evaluated using different solvents, no solvent effect on mutagenicity was observed. The investigators concluded that no clear relationship existed between the solvent used and the mutagenicity observed (Katz et al. 1981).

Carcinogenicity

Gum Arabic

No evidence of carcinogenicity was noted in rats dosed intraperitoneally with gum arabic (1.75% or 7% in saline or water) three times per week for up to 15 weeks. Based on the data presented, it was difficult to ascertain the size of the dose administered. The doses administered were on the order of several hundred mg/kg. Also, no evidence of carcinogenicity was found in a similar study using mice (doses injected not stated) (FASEB 1973).

Gum arabic gruel was injected intramediastinally (single dose) into five (0.5 ml dose of test substance) and 10 (1 ml dose) guinea pigs. The animals (strain not specified) ranged in weight from 220 to 450 g and were 4 to 10 months old. Neoplasms were not observed in any of the guinea pigs either at necropsy or at microscopic examination of tissue. On the average, the animals survived from 1200 to 1490 days (Tlolka-Pluszczyk 1970).

Melnick et al. (1983) studied the carcinogenicity of gum arabic using 4-week-old F344 rats (50 males, 50 females) and

TABLE 10
In vitro and in vivo mutagenicity of Gum Arabic, as Gum Arabic, Acacia, and Gum Acacia

Test substance	Strains/cells/animals tested	Test procedure	Test results	References
		Gum Arabic		
Bacterial Cell Test Systems				
Gum Arabic	*Saccharomyces cerevisiae* D4	Host-mediated assay for mitotic recombination (Gabridge and Legator 1969); test concentration of 5% w/v if no lethal effects observed	Not mutagenic	Maxwell and Newell 1974
Gum Arabic	*Salmonella typhimurium* G-46 and TA-1530	Ames test (Ames 1971)	Not mutagenic	Maxwell and Newell 1974
Gum Arabic (in DMSO)	*Salmonella typhimurium* TA1535, TA1537, and TA1538 *Saccharomyces cerevisiae* D4	Plate and suspension assays with and without metabolic activation. Plate test concentrations up to 3.3%. Suspension assay concentrations up to 0.36%	Not mutagenic with or without metabolic activation	Litton Bionetics, Inc. 1975
Gum Arabic	*Saccharomyces cerevisiae* D3	Plate test (Brusick 1973)	Not mutagenic	Green 1977
Gum Arabic (in 0.067 M sodium phosphate buffer)	*Salmonella typhimurium* TA1535, TA1537, TA1538, TA98, and TA 100 *E. coli* WP2 (uvrA)	*Salmonella*/microsome assay with and without metabolic activation; concentrations up to 10,000 μg/plate	Not toxic or mutagenic	SRI International 1980
Gum Arabic (in water)	*Bacillus subtilis* M 45 Rec^- and H 17 Rec^+	Spore rec-assay (with and without metabolic activation) for DNA-damaging activity	Not mutagenic	Ishizaki and Ueno 1987
Gum Arabic (in 0.067 M potassium or phosphate buffer)	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, and TA1538 *Escherichia coli* WP2	*Salmonella* strains tested in plate incorporation assay (Ames et al. 1975) with and without metabolic activation; doses up to 10 mg/plate. *E. coli* tested according to modification of plate incorporation assay at same doses	Not mutagenic with or without metabolic activation	Prival et al. 1991
Gum Arabic	*Salmonella typhimurium* TA100, TA1535, TA1537, TA97, and TA98	Modification of preincubation procedure by Haworth et al. (1983) with and without metabolic activation. Cultures incubated with 0.05 ml gum arabic	Not mutagenic with or without metabolic activation	Zeiger et al. 1992
Mammalian Cell Test Systems				
Gum Arabic	Diploid human embryonic lung (WI-38) cells	Cytogenetics assay; concentrations up to 1000 μg/ml culture if no cytotoxicity observed at this level. Anaphase analyses according to procedure of Nichols et al. (1971)	Response classified as “slight positive.” No definite abnormal anaphase figures observed	Maxwell and Newell 1974

(Continued on next page)

TABLE 10

In vitro and in vivo mutagenicity of Gum Arabic, as Gum Arabic, Acacia, and Gum Acacia *(Continued)*

Test substance	Strains/cells/animals tested	Test procedure	Test results	References
Gum Arabic	WI-38 human embryonic lung cells	Test methodology not stated	Chromosomal aberrations induced in anaphase	Green 1977
Animal Test Systems				
Gum Arabic	Male albino rats (weights 200 g)	Acute and short-term in vivo cytogenetics assays. Doses up to maximum tolerated dose administered. Cytogenetic evaluations on bone marrow cells in metaphase	No significant positive response, but may have been a slight positive response. Further tests and detailed statistical evaluation needed to confirm this possibility	Maxwell and Newell 1974
Gum Arabic	Male and female Swiss mice (10 to 12 weeks old; weights = 25 to 30 g)	Dominant lethal test. Male mice dosed orally with 1% gum arabic prior to mating	No dominant lethal effect	Kar et al. 1984
Gum Arabic	NMRI mice (weights between 30 to 35 g)	Micronucleus test (bone marrow smears). Mice dosed i.p. with 3% gum arabic	Not genotoxic	Wild et al. 1985
Gum Arabic	Male NMRI mice (weights between 30 to 35 g)	Intrasanguineous host-mediated assay. *Salmonella typhimurium* strain TA 98 culture (0.1 ml) injected into tail vein. Intravenous injection followed by oral dose of 3% gum arabic	Not mutagenic to strain TA 98	Wild et al. 1985
	C57BL virgin female mice	Mouse coat color spot test (transplacental mutagenicity test). Gum Arabic (3%) injected i.p. after mating. Spots classified as relevant caused by mutations at heterozygous coat-color loci	Not mutagenic	
	C57BL mice	Mouse melanocyte test—Used to detect somatic mutations that affect the morphology of pigment cells. Pregnant females received i.p. injections of 3% gum arabic on 16th day after detection of vaginal plug	Not genotoxic	
Gum Arabic	Male and female Sprague-Dawley rats (males: 6 to 8 weeks old; females: 10 to 12 weeks old)	Dominant lethal test. Male rats fed concentrations up to 4% w/w gum arabic prior to mating. Number of live and dead implants counted 14 days after midweek of mating	Statistically significant dominant lethal effects in male rats	Sheu et al. 1986

	(SEC × C57BL)F1 and (C3H × C57BL)F1 female mice (10 to 12 weeks old)	Dominant lethal test. Male mice fed diets containing up to 20% gum arabic prior to mating	No evidence of dominant lethal effect	
	(101 × C3H)F1 male mice (8 weeks old)	Heritable translocation test. Male mice fed test diet containing 15% *w/w* gum arabic prior to mating	No reduction in average litter size. Number of translocation-carrying male progeny in test group was comparable to that of control group	
		Acacia		
Animal Test Systems				
Acacia (in water)	Male Swiss-Webster mice (6 weeks old; mean weights between 16 to 32 g)	Micronucleus test (bone marrow smears). Mice dosed with 2% Acacia in water	Not genotoxic	MacGregor et al. 1983
Acacia	Inbred female Chinese hamsters (*Cricetulus griseus*) (weight range, 26 to 32 g)	Assay for sister chromatid exchanges. Hamsters dosed i.p. or orally with 10% Acacia (dose volume = 10 ml/kg)	Mean number of sister chromatid exchanges not significantly different, compared to control hamsters dosed with 0.9% normal saline	Neal and Probst 1983
Gum Acacia	Male Swiss mice (6 to 8 weeks old)	Chromosomal aberrations and sperm-head morphology assays. Mice dosed with 5% Gum Acacia by gavage (volume per dose = 0.5 ml)	No statistically significant differences in frequency of chromosomal aberrations and incidence of sperm head abnormalities, compared to control (distilled water) group	Prasad et al. 1987
Gum Acacia	Male Swiss albino mice (8 weeks old)	Micronucleus test (bone marrow smears). Mice dosed orally with 5% Gum Acacia	The ratio of polychromatic erythrocytes to monochromatic erythrocytes (P/N ratio) was slightly higher, compared to mice dosed with water	Pentiah et al. 1989
Acacia	Male ICR mice (7 weeks old; weights between 28 and 32 g)	Micronucleus test (bone marrow smears). Mice dosed with 10% Acacia by gavage (volume per dose = 0.02 ml/g body weight	Not genotoxic	Parton et al. 1988
Acacia	Male ICR mice	Micronucleus test (bone marrow smears). Mice dosed with 10% Acacia by gavage (volume per dose = 20 ml/kg)	Not genotoxic	Parton et al. 1990

4- to 5-week-old B6C3F$_1$ mice (50 males, 50 females) in a 2-year chronic study. Both male and female rats were divided into high- and low-dose groups. Low-dose animals were fed gum arabic at a concentration of 25,000 ppm in the diet and high-dose animals were fed 50,000 ppm. Test diets were fed for 103 consecutive weeks, followed by 1 to 2 weeks of feeding of the basal diet. Control mice (50 males, 50 females) and rats (50 males, 50 females) were fed the basal diet only according to the same schedule. Moribund animals and animals that survived to the end of the study were killed using carbon dioxide and necropsied. Tissues were preserved for histopathologic evaluation.

Changes in mean body weight for male and female rats were comparable to those of the respective control groups throughout the study. Slight decreases in body weight (7% to 13%) were observed in female rats. Compared to controls, consistent differences in mean body weight were noted for female mice of the high dose group (50,000 ppm in diet). No significant differences were found in survival between experimental mice or rats when compared to the respective control groups.

Neoplasms were observed only in male rats, and were diagnosed as malignant lymphomas or leukemia/lymphoma. The incidences of malignant lymphomas for control, low-dose (25,000 ppm gum arabic), and high-dose (50,000 ppm gum arabic) experimental groups of male rats were as follows: 4/50 (low-dose), 1/50 (high-dose), 8/50 (concurrent controls), and 31/1066 (historical controls). Compared to the concurrent control group, a significant decrease ($p < .05$) in tumor incidence was observed in the high dose group, and this was the only statistically significant finding for this neoplasm.

The incidences of neoplasms classified as leukemia/lymphoma in control, low-dose (25,000 ppm gum arabic), and high-dose (50,000 ppm gum arabic) groups of male rats were: 19/50 (low-dose), 16/50 (high-dose), 18/50 (concurrent controls), and 238/1066 (historical controls). Compared to concurrent controls, no statistically significant differences were observed in the incidence of tumors of this type.

No significant changes were observed in the incidence of primary neoplasms in mice that were fed gum arabic in the diet at concentrations of 25,000 or 50,000 ppm. Based on the preceding results, the investigators concluded that gum arabic was not carcinogenic in F344 rats or B6C3F$_1$ mice of either sex (Melnick et al. 1983).

Cocarcinogenicity

Gum Arabic

Vogel and Zaldivar (1971) studied the cocarcinogenicity of Gum Acacia using male rats of the Buffalo strain (6 to 10 weeks old). Thirty-four rats were exposed to fission neutrons (single exposure of 300 to 364 rads; whole-body irradiation), followed by three intraperitoneal injections (0.5 ml per injection) of a 7% solution of Gum Acacia in 0.85% sodium chloride weekly for 23 weeks.

A second test group (30 rats) was irradiated after treatment with Gum Acacia according to the same procedure. Three groups of rats served as controls: one of the control groups (50 rats) was exposed to fission neutrons only. Two additional control groups consisted of 40 rats injected intraperitoneally with 7% Gum Acacia only (according to test group protocol) and an untreated control group of 79 rats.

No significant neoplasm incidence was present in the two control groups. However, the survival time for the 40 control rats injected with Gum Acacia (554.8 $\pm$ 39.4 days, $n = 30$) was significantly shortened when compared to untreated controls (669.2 $\pm$ 19.0 days, $n = 58$). Increases in hepatic, gastric, and intestinal neoplasms were noted in the first test group (34 rats; neutron exposure followed by Gum Acacia injections), when compared to the group of 50 rats exposed to fission neutrons only.

Except for gastric neoplasms, these differences in neoplasm incidence were considered small and probably not significant. It is important to note that no gastric neoplasms were observed in the 50 rats exposed to fission neutrons only, whereas, 20% of the 34 test rats had gastric cancers. No explanation for this difference was given.

Tissues of 28 of the 34 test rats in this group were subjected to complete histopathological analysis after necropsy. Similarly (compared to fission neutrons control group), no gastric neoplasms were noted in the group of 30 rats treated with Gum Acacia and then exposed to fission neutrons. The investigators stated that this finding could have been due to the small number of rats ($n = 14$, compared to $n = 28$ in other test group) subjected to complete histopathological examination after necropsy.

The authors stated that the data presented in this study suggest that Gum Acacia might be considered a "potentiator" for carcinogenesis (Vogel and Zaldivar 1971).

Gum arabic has been reported to increase the number of metastases in mice injected intraperitoneally with Ehrlich ascites carcinoma cells. The carcinoma cells were injected 6 or 24 h after the mice were injected intravenously with gum arabic. However, under some conditions, ascites tumor formation was inhibited (Osswald 1968).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Gum Arabic

Studies on the reproductive and developmental toxicity of gum arabic are summarized in Table 11 and discussed below.

The antifertility activity of Gum Acacia (1 ml in water) was evaluated using 10 female rats (strain and weights not stated). The test substance was administered by stomach tube daily for a period of 5 days after mating. After performing laparotomy on anesthetized dams, the number of fetuses was counted on the 10 day of pregnancy. The average number of implants per rat was 7.8. The percentage of rats with no implant was 0 (Sabir and Razdan 1970).

In a study by the Food and Drug Research Laboratories (1972), the teratogenicity of gum arabic was evaluated using six

TABLE 11
Reproductive and developmental toxicity studies

Test substance	Animals/cells tested	Test procedure	Test results	References
		Acacia		
10% aqueous Acacia solution	9 Little Dutch female rabbits (average weight between 2.1 kg)	After mating, 10% aqueous Acacia solution administered orally on day 0 and the following 6 days	Normal microscopic variations in blastocysts reported: minor trophoblastic vacuolation, trophoblastic degeneration granules, and trophoblastic knob formations	Schardein et al. 1965
Gum Acacia	10 female rats	Gum Acacia (1 ml in water) administered orally during 5-day period after mating	No antifertility activity. Average number of implants per rat = 7.8	Sabir and Razdan 1970
Gum Acacia	Two groups of 5 male albino Wistar rats (4 months old; weights between 180 to 200 g)	First group dosed orally (dose = 1 ml) daily for 24 days. Second group dosed orally (dose = 1 ml) for 48 days	No suppression of spermatogenesis	Akbarsha and Manivannan 1973
4% Gum Acacia	6 Haffkine albino rabbits (weights between 175 to 225 g)	Males dosed orally daily for 28 days and mated with untreated females for total of 12 weeks	No statistically significant difference in number of pregnant females. No antifertility effect in males	Yegnanarayan and Joglekar 1978
	Adult female rabbits (weights between 1 to 2 g)	Dosed orally with 4% Gum Acacia for two days	No inhibitory effect on ovulation	
	Female albino rats (weights between 150 to 200 g)	4% Gum Acacia administered orally to 10 females over period of two estrus cycles, followed by mating with males during proestrus phase of third estrus cycle (short-term experiment). 4% Gum Acacia administered orally to 6 females over period of 6 estrus cycles, followed by mating during proestrus stage of 7th estrus cycle	No significant differences in mating (number of females inseminated) between experimental and control groups. No significant changes in duration of estrus cycles after dosing	

(Continued on next page)

TABLE 11
Reproductive and developmental toxicity studies *(Continued)*

Test substance	Animals/cells tested	Test procedure	Test results	References
	10 female rats (weights between 150 to 200 g)	Females dosed orally with 4% gum arabic on days 1 to 7 of pregnancy	No statistically significant difference in average litter sizes between experimental and control groups, indicating that fetal resorption did not occur	
	10 female rats (weights between 150 to 200 g)	Females dosed orally with 4% gum arabic on days 10 to 16 of pregnancy	No statistically significant differences in number of pups delivered between experimental and control groups	
5% Gum Acacia	9 Syrian golden hamsters (8 weeks old; weights between 80 to 100 g)	Dosed orally with 5% Gum Acacia (dose volume = 0.1 ml/10 g body weight) daily for 54 days	All of the hamsters produced morphologically normal sperm	Waller et al. 1983
1% Gum Acacia	10 female Charles Foster rats (90 days old; weights between 200 ± 20 g)	Administered daily at dose of 50 mg/kg/day during the period of organogenesis	No gross or visceral defects	Sethi et al. 1989
		Gum Arabic		
1% aqueous suspension or mucilage prepared from gum arabic	NMRI mice	1% aqueous suspension or mucilage prepared from gum arabic injected intraperitoneally (single injection or series of 5 injections), subcutaneously (5 injections), and administered orally (5 times) between the 11th and 15th day of gestation	No lethal effects on fetuses	Frohberg et al. 1969
Gum Arabic	Adult female albino CD-1 outbred mice (4 groups). Most groups contained 22 to 23 mice	The four groups of mated mice received oral doses of 16, 75, 350, and 1600 mg/kg on days 6 through 15 of gestation	The number of abnormalities observed in soft or skeletal tissues of fetuses did not differ from the number occurring spontaneously in sham-treated controls	Food and Drug Research Laboratories 1972
	Groups of female rats, rabbits, and hamsters	Oral doses of 16, 75, 350, and 1600 mg/kg on gestation days 6 through 10 (hamsters) and 6 through 15 (rats). Oral doses of 8, 37, 173, and 800 mg/kg in corn oil on days 6 through 18 of gestation (rabbits)	The number of abnormalities observed in soft or skeletal tissues of fetuses did not differ from sham-treated controls	

Gum Arabic (Acacia Senegal Gum)	Groups of 4-week-old Osborne-Mendel (FDA strain) rats	Groups fed dietary concentrations up to 15% beginning at week 13 prior to mating	Gum Arabic not classified as a reproductive or developmental toxicant in rats	Collins et al. 1987
5% aqueous gum arabic solution	36 female Sprague-Dawley Crl:CDBR rats (~9 months; weights between 207 to 314 g)	Solution administered orally once daily (5 ml/kg/day) on days 6 through 17 of gestation	External, visceral, and skeletal malformations observed were unrelated to dosing with Acacia	Morseth and Ihara 1989a
5% aqueous gum arabic solution	30 male Sprague-Dawley Crl:CDBR male rats (6 weeks old; weights between 181.9 to 226.3 g) 30 female rats of same strain (10 weeks old; weights between 210.9 to 309.9 g)	Solution administered orally to females once daily (5 ml/kg/day) for 14 days prior to mating, throughout the mating period, and through day 19 of gestation or day 21 of lactation. Solution also administered to males prior to and during mating and until animals killed	No treatment-related abnormal estrous cycles. No external, skeletal, or soft tissue malformations	Morseth and Ihara 1989b
Gum Arabic	12 Sprague-Dawley rats (adult males)	Control rats fed 30% gum arabic in the diet for 82 days	No effect on spermatogenesis (all males were fertile)	Huynh et al. 2000

groups of mated adult female albino CD-1 outbred mice. Three of the test groups consisted of 22 to 23 mice per group and received doses of 16, 75, and 350 mg/kg, respectively, on days 6 through 15 of gestation. Doses were administered by oral intubation. The fourth test group of 31 mice was dosed with gum arabic (1600 mg/kg) according to the same procedure. Sham-treated mice (28) served as negative controls, and positive-control mice were dosed with aspirin (150 mg/kg). Mean body weights for the test groups ranged from 30 to 39.7 g and were 31.2 g and 31.8 g for negative and positive controls, respectively.

On day 17, all dams were placed under anesthesia and cesarean section was performed. The numbers of implantation sites, resorption sites, and live and dead fetuses were recorded. The urogenital tract of each dam was examined in detail for anatomical normality. Gross examinations for the presence of external congenital abnormalities were performed on all fetuses. Detailed visceral examinations employing 10× magnification were performed on one-third of the fetuses from each litter. The remaining two-thirds were examined for skeletal defects.

The administration of gum arabic to pregnant mice at doses up to 1600 mg/kg had no clearly discernible effect on nidation or maternal or fetal survival. The number of abnormalities observed in either soft or skeletal tissues of fetuses from test groups did not differ from the number occurring spontaneously in sham-treated controls.

As part of this study, groups of rats, rabbits, and hamsters were dosed with gum arabic according to the following modifications of the above test procedure: doses (indicated above) were administered to hamsters (gestation days 6 through 10), rats (gestation days 6 through 15), and rabbits (gestation days 6 through 18). Cesarean sections were performed earlier on hamsters (day 14) and later on rats (day 20). Positive-control rats and hamsters received a higher dose of aspirin (250 mg/kg). Rabbits were dosed with gum arabic in corn oil (8, 37, 173, and 800 mg/kg, respectively); cesarean sections were performed on day 29. Rabbits were injected with human chorionic gonadotropin (day 0) and artificially inseminated. Mean weights for the dams tested were as follows: 200 to 216 g (24 rats per group), 104.6 to 118.4 g (21 to 24 hamsters per group), and 2.01 to 2.43 kg (15 rabbits per group).

The administration of gum arabic, in corn oil, to pregnant rabbits at doses up to 37 mg/kg (highest dose tested = 800 mg/kg) had no clear effect on nidation or maternal or fetal survival. The number and types of abnormalities observed in fetal soft or skeletal tissues from this group did not differ from the number occurring spontaneously in the sham-treated controls. Of the four test groups of rabbits (15 dams per group), the number of survivors per dose group was reported as follows: 13 rabbits (8.0 mg/kg dose group), 15 rabbits (37.0 mg/kg), 12 rabbits (173.0 mg/kg), and 9 rabbits (800.0 mg/kg). In 173 and 800 mg/kg dose groups, maternal death was preceded by severe bloody diarrhea, urinary incontinence, and anorexia. At necropsy, hemorrhage in the mucosa of the small intestines was the only gross pathological finding (Food and Drug Research Laboratories 1972).

Akbarsha and Manivannan (1993) studied the reproductive toxicity of Gum Acacia using two groups of five male albino rats of the Wistar strain (4 months old; weights between 180 and 200 g). The test substance was administered orally (dose = 1 ml) to the first group daily for 24 days. The second group was dosed (dose = 1 ml) daily for 48 days. Rats in both groups were necropsied 24 h after the last dose.

The testis, epididymis (divided into caput and cauda), seminal vesicle, ventral prostate, and coagulating gland were excised, homogenized, and centrifuged. The supernatant was used for determination of total protein and acid phosphatase (ACPase) and alkaline phosphatase (ALPase) activities. Supernatant obtained from the testes was also used for the determination of glycogen and cholesterol, and lactate dehydrogenase (LDH) activity.

The authors stated that increased glycogen and LDH in the testis are both consequences of spermatogenic arrest, and that decreased ACPase and increased ALPase activities in the testis also reflect the suppression of spermatogenesis. They concluded that Gum Acacia did not suppress spermatogenesis in this study (Akbarsha and Manivannan 1993).

Huynh et al. (2000) used gum arabic as the vehicle control in a study evaluating the effect of triptolide (deterpene triepoxide) on spermatogenesis in adult male Sprague-Dawley rats (12 animals, 90 days old). Control males were fed 30% gum arabic in the diet daily for 82 days. Males in the test group were each fed triptolide at a daily dose of 100 μg/kg body weight. Male and female rats (two females per male) were housed together during the feeding period, after which pregnancy rates were determined. The presence of sperm in morning vaginal smears was used to determine whether or not mating was successful. Any male that impregnated at least one of the females was considered fertile. All 12 control males were fertile, whereas all males fed triptolide in the diet were sterile.

Collins et al. (1987) evaluated the teratogenicity of gum arabic (Acacia Senegal Gum) using groups of 4-week-old Osborne-Mendel (FDA strain) rats. Beginning at 13 weeks prior to mating, the rats were fed gum arabic at concentrations of 1%, 2%, 4%, 7.5%, or 15%, respectively. Another group of rats was fed a control diet. Control and test diets were also fed throughout mating and gestation. After mating was confirmed, females were placed in groups of 41 to 47. The dams were killed on day 20 of gestation.

One female rat (1% dietary group) died during the study. External observations of the dams were unremarkable. One female (7.5% dietary group) did have a cystic ovary and one had lung nodules (15% dietary group). Sporadic nonsignificant increases in body weight were observed in all experimental groups.

The percentage of pregnant females was approximately the same in all experimental groups and controls. Mean numbers of corpora lutea and implants per female were also similar to control values, and the average number of viable fetuses was similar in all groups. No effect was seen in any group with respect to the mean number of viable males and females. Three litters were totally resorbed, one litter from the control, 1%, and 4% dietary groups. Gum arabic in the diet had no effect on the percentage of females with at least one resorption or with at least two resorptions. The numbers of early and late deaths, singly or combined (as average percentage of resorptions), were similar to control values.

The feeding of gum arabic had no effect on mean fetal body weights and crown-rump lengths. The ingestion of gum arabic also had no effect on the distribution of fetuses by sex. A significant decrease in mean female body weight in the 1% dietary group was noted; however, this observation was deemed a random occurrence. The significant increase in the length of females in the 4% and 7.5% dietary groups was not considered biologically significant.

The investigators stated that because of the large group of animals in this study, small variations in crown-rump length can result in significant effects. Similar numbers of runts were noted among male and female fetuses from all dietary groups, with the exception of no runts among male fetuses in the 1% and 15% dietary groups.

Regarding external variations in live fetuses, spina bifida and exencephaly were observed in two fetuses from the control group. No other terata were observed, and the external variations were distributed randomly. Similar numbers of fetuses with hemorrhages were observed in all dietary groups.

The mean numbers of sternebral variations per litter varied from 4.18 (4% gum arabic dietary group) to 5.09 (15% dietary group) in experimental groups, and the mean number of sternebral variations per litter in the control group was 5.21. The variations included reduced ossification and bipartite, missing, and malaligned sternebrae. No dose-related increases were found with respect to any of the observed sternebral deficiencies, and no significant differences were found between experimental and control groups. The significant decrease in the average number of fetuses with one or more sternebral variations per litter that was observed in the 4% and 7.5% dietary groups was considered a random occurrence. Thus, the ingestion of gum arabic did not affect the incidence of litters with fetuses with sternebral variations.

Skeletal ossification deficiencies were observed in bones other than sternebrae; however, no dose-related differences were observed between experimental and control groups with respect to any variation. Furthermore, no dose-related effect was found on the incidence of variations, fetuses with variations, or litters affected in any of the dietary groups.

Also, no dose-related effect was observed on the incidence of any type of soft-tissue variation. Most of the soft tissue variations involved the kidneys. Additionally, the incidence of soft tissue variations in fetuses from experimental and control groups was similar. The mean numbers of soft tissue variations per litter ranged from 0.30 (15% dietary group) to 0.82 (7.5% dietary group), and the mean was 0.76 per litter in the control group (Collins et al. 1987).

Schardein et al. (1965) administered a 10% aqueous *Acacia* solution by gavage to two groups of nine Little Dutch strain mated female rabbits (average weight = 2.1 kg) at doses of 1.26 and 1.5 ml/kg, respectively. Doses were administered on day 0 and the following 6 days (7 doses per female). Nine untreated rabbits served as negative controls. Blastocysts were removed from the uterine horns at 6.5 days of age, prepared as flat mounts, and then evaluated.

The number of fertile rabbits with blastocysts recovered (eight of nine rabbits) in the 1.26 and 1.5 ml/kg dose groups was the same as that noted for the untreated control group. The mean numbers of blastocysts per rabbit were as follows: untreated controls (5.3 ± 1.2), 1.26 ml/kg dose group (7.0 ± 1.7), and 1.5 ml/kg dose group (5.4 ± 2.2). Normal microscopic variations in blastocysts were reported for test and control groups. These variations included minor trophoblastic vacuolation, trophoblastic degeneration granules, and trophoblastic knob formations (Schardein et al. 1965).

Morseth and Ihara (1989a) studied the teratogenicity of a 5% solution of gum arabic (powder) in distilled water using 36 female Crl: CDBR rats (~9 months old) for which mating had been confirmed. Body weights on gestation day 0 ranged from 207 to 314 g. The solution was administered by gavage once daily (5 ml/kg/day) on gestation days 6 through 17. The dams were necropsied on day 20 of gestation. Fetuses were subjected to external (303 fetuses), visceral (102 fetuses), and skeletal (201 fetuses) examinations.

External variations were not observed in any of the fetuses evaluated; however, external malformations, brachygnathia and rudimentary/short tail, were observed in one fetus. Visceral variations included only two fetuses with increased renal pelvic cavitation. At skeletal evaluation, one fetus had brachygnathia, tail short/rudimentary, abnormal fusion of sternebrae, and vertebral anomaly with/without associated rib anomaly. The external, visceral, and skeletal malformations observed were unrelated to dosing with Acacia (Morseth and Ihara 1989a).

Morseth and Ihara (1989b) evaluated the effect of a 5% solution of gum arabic (powder) in distilled water on fertility and general reproductive performance using 30 male (6 weeks old; weights = 181.9 to 226.3 g) and 30 female (10 weeks old; weights = 210.9 to 309.9 g) Sprague-Dawley Crl: CDBR rats. The solution was administered (oral intubation) to male and female rats once daily (5 ml/kg/day) for 63 days prior to mating, throughout the mating period, and until the animals were killed. Male rats were killed after the females had littered. The oral dosing schedule for female rats was daily for 14 days prior to mating, throughout the mating period, and through gestation day 19 or day 21 of lactation. Fifteen female rats were killed on day 20 of gestation, and the remaining females were allowed to raise their neonates to day 22 postpartum.

No abnormal estrous cycles that were considered treatment-related were observed in any of the females. Twenty-nine of the 30 females became pregnant; the male fertility index was 97%. Mean viability and mean weaning indices were 96% and 98%, respectively. No external, skeletal, or soft tissue malformations were observed (Morseth and Ihara 1989b).

The reproductive toxicity of 5% Gum Acacia was evaluated using nine male Syrian golden hamsters (8 weeks old; weights = 80 to 100 g). The males were mated with female Syrian golden hamsters in order to confirm fertility. Subsequently, the males were dosed (oral gavage) with 5% Gum Acacia (dose volume = 0.1 ml/10 g body weight) daily for 54 days. The animals were killed 3 days after the last dose. As determined by analysis of testis sections, spermatogenesis was reported for all hamsters. All of the hamsters produced morphologically normal sperm, which were also observed in the epididymis (Waller et al. 1983).

Yegnanarayan and Joglekar (1978) studied the antifertility effects of 4% Gum Acacia in a series of five experiments using male and female rats and female rabbits of the Haffkine strain.

In the first study, six male albino rats (weights = 175 to 225 g) were tested. The rats were dosed orally daily for 28 days using a rubber catheter. Beginning on the first day of feeding, males were mated (one male to two females) with females for 12 weeks. Females were replaced each week of feeding. Additional groups of females were mated with control males dosed with saline according to the same procedure. Vaginal smears were examined daily for the presence of spermatozoa. Pregnant females were surgically observed on the tenth day of pregnancy.

The number of inseminated females (73) was the same in experimental and control groups. The total number of pregnant females in experimental and control groups was 24 and 37, respectively, but this difference was not statistically significant.

In the second experiment, the effect of 4% Gum Acacia on the estrus cycle and mating was evaluated using fertile female albino rats (weights = 150 to 200 g). The experiment was divided into two phases. In the first phase (short-term treatment), 4% Gum Acacia was administered orally to 10 female rats over a period of two estrus cycles, beginning on the day of proestrus. The females were mated singly with males during the proestrus phase of the third estrus cycle. In the second phase (long-term treatment), 4% Gum Acacia was administered orally to six female rats over a period of six estrus cycles, beginning in the proestrus phase. Mating was allowed in the proestrus stage of the seventh estrus cycle. In both the first and second experimental phases, control females dosed with saline were mated with males according to the same procedures, respectively. Results for the first and second phases of this experiment indicated no significant differences in mating (number of females inseminated) between experimental and control groups. Additionally, for both phases, no significant changes were observed in the duration of estrus cycles after dosing.

The third experiment, for determining anti-implantation effects, involved 10 fertile rats (weight range from 150 to 200 g) that were mated in proestrus singly with fertile males. Females were dosed orally with 4% gum arabic on days 1 to 7 of pregnancy. The animals were allowed to deliver normally and litter sizes were recorded. Ten control females dosed with saline were mated according to the same procedure. No statistically significant differences were observed in average litter sizes between experimental and control groups, indicating that fetal resorption did not occur in litters of rats dosed with 4% gum arabic.

The fourth experiment was performed to determine any postimplantation effect of 4% gum arabic using ten fertile rats (weights = 150 to 200 g). Female rats were dosed orally with 4% gum arabic on days 10 to 16 and the number of pups delivered was determined. The rats were observed for vaginal bleeding, indicative of abortifacient activity during pregnancy. Control females were dosed with saline according to the same procedure. One of 10 experimental rats did not have a litter. All control females had litters. No statistically significant differences were observed in the number of pups delivered between experimental and control groups.

In the fifth experiment, the antiovulatory potential of 4% Gum Acacia was evaluated using adult female rabbits (number not stated; weights = 1 to 2 kg). The rabbits were dosed orally with 4% gum arabic for 2 days. Copper acetate (4 mg/kg) was then injected into the marginal ear vein in order to induce ovulation. At 48 h post injection, laparotomy was performed; fresh bleeding points on the ovaries were indicative of ovulation. Control rabbits were pretreated with saline according to the same procedure prior to the injection of copper acetate. After the injection of copper acetate, bleeding points on the ovaries were observed in all control and experimental rabbits. Therefore, the authors concluded that 4% Gum Acacia did not have an inhibitory effect on ovulation (Yegnanarayan and Joglekar 1978).

A 1% aqueous suspension or mucilage prepared from gum arabic had no lethal effects on fetuses of NMRI mice injected intraperitoneally (single injection or series of five injections), subcutaneously (five injections), or administered orally (five times) between the 11th and 15th day of gestation (Frohberg et al. 1969).

The embryotoxicity of 1% Gum Acacia was evaluated using ten Charles Foster rats (90 days old; weights = 200 ± 20 g). The test substance was administered daily at a dose of 50 mg/kg/day during the period of organogenesis. The fetuses were delivered by cesarean section on day 20 of gestation, fixed in Bouin's solution, and examined for visceral and skeletal defects. None of the fetuses had gross or visceral defects (Sethi et al. 1989).

CLINICAL ASSESSMENT OF SAFETY

Absorption, Distribution, and Excretion

Gum Arabic

The FASEB (1973) review stated that there was no evidence of the absorption of intact gum arabic found in a study using infants. Twenty-two infants, 1 to 15 months old, were fed gum arabic (15 to 20 g per day) in milk. No urinary excretion of pentose or significant excretion of gum arabic was observed in the stools.

In a nephrotic patient, 20% of the gum arabic injected intravenously over a period of 6 weeks was excreted in the urine.

Other studies involving patients with nephrosis indicated that intravenously injected Gum Acacia, or some product associated with it, accumulated in the liver and remained in the tissues for several months. Serious disturbances in hemoglobin, white blood cells, and serum proteins, all nonlethal effects, were noted (FASEB 1973).

Ross et al. (1984a) evaluated the excretion of gum arabic and its effect on glucose absorption and routine hematological and biochemical measurements in five healthy male volunteers (30 to 55 years old). All subjects were free of signs of gastrointestinal disease. The study was divided into two time periods, a 7-day control period that was followed by a 24-day treatment period. After an overnight fast, glucose (50 g in 200 ml H_20) was fed to each subject on the first day of the control period. During the 24-day treatment period, gum arabic (25 g in 125 ml 7% dextrose) was ingested daily by each subject. Urine was collected on 1 day of the control period and on 1 day during the 3rd week of the treatment period. Complete 5-day fecal collections were made on days 2 to 6 of the control period and on days 16 to 20 of the treatment period. Pooled stool slurry samples from the five subjects were centrifuged. A precipitate typical of gum arabic was not detected in fecal specimens collected before or after the administration of gum arabic.

The marked increases in breath hydrogen production noted after gum arabic ingestion were indicative of bacterial breakdown of gum arabic in the cecum and colon after 3 weeks of administration. Additional study results are summarized in the following paragraph.

No significant differences in the mean concentration of serum lipids (phospholipids and triglycerides) were noted before and after gum arabic ingestion. However, a significant decrease in serum cholesterol (0.39 mmol/L reduction; $p < .05$) was noted. Also, no statistically significant differences were observed between the mean blood glucose concentration (control) and the glucose concentration after the administration of gum arabic.

Similarly, no significant differences were found in the mean insulin concentration (before versus after gum arabic ingestion). Alanine aminotransferase and aspartate aminotransferase activities were significantly reduced ($p < .0025$; $p < .001$) after gum arabic ingestion; however, both mean values were within the normal limits for the population. Of the 13 biochemical measurements that were estimated in the plasma, these reductions in plasma enzyme concentrations represented the only noted significant changes (Ross et al. 1984a).

Short-Term Oral Toxicity

Gum Arabic

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

Short-Term Intravenous Toxicity

Acacia (Not Gum Arabic)

Acacia was administered to nine patients with nephrotic edema over periods up to 8 weeks. The test substance was administered intravenously, and total doses ranged from 80 to 325 g. No signs or symptoms of hepatic enlargement or any other complications were observed. Five of the patients excreted 5.5% to 38% of a single dose in the urine during periods ranging from 10 to 30 days, respectively (World Health Organization 1974).

Skin Irritation

Acacia (Not Gum Arabic)

The skin irritation potential of Acacia Farnesiana Extract (from flowers, 4.0% in petrolatum) was evaluated in a 48-h closed-patch test using 30 healthy male and female volunteers. Skin irritation was not observed (Letizia et al. 2000).

Shaligram and Vakil (1990) evaluated the skin irritation potential of Acacia Concinna Fruit Extract (2% in carageenan base [pH of 6 to 7] or 2% in a shampoo [pH of 7 to 8]) in a use test involving 30 normal subjects. The carageenan base and the shampoo, both without the fruit extract, served as controls. The application procedure was described as a routine half head (wet surface) application of Acacia Concinna Fruit Extract (in carrageenan base or in shampoo). The respective controls were applied to the other half of the head (wet surface). Application of test and control materials was followed by rinsing with warm water at 10 to 15 min post exposure. The scalp of each subject was evaluated for signs of irritation (erythema, edema, or any other reaction) at 24 and 48 h post application.

Neither Acacia Concinna Fruit Extract (2% in carrageenan or 2% in shampoo base) nor the controls induced skin irritation (Shaligram and Vakil 1990).

Skin Sensitization

Gum Arabic

Ivy Laboratories (2000) evaluated the skin sensitization potential of a mascara containing 8.0% Acacia Senegal in a maximization test using 28 healthy adult volunteers (males and females, 18 to 49 years old). Twenty-five subjects completed the study because three withdrew for reasons that were unrelated to the test procedure. During the induction phase, approximately 0.1 ml of 0.25% aqueous sodium lauryl sulfate (SLS) was applied (under an occlusive patch) to each subject. Patches were applied to the upper outer arm, volar forearm, or to the back for 24 h. After patch removal, 0.1 ml of the mascara was applied (under an occlusive patch) to the same site on each subject. Patches remained in place for 48 h, except for weekend applications in which the contact period was 72 h. Sites were observed for signs of irritation at the time of patch removal.

If skin irritation was not observed, an occlusive patch containing 0.25% aqueous SLS was applied to the same test site for 24 h. An occlusive patch containing the test substance was then applied to the same site for 48 h. The preceding patch application

TABLE 12
Case reports on Gum Arabic and other species of Acacia

Ingredient studied	Patients evaluated	Procedure/route of exposure	Results	Reference
Acacia	78-year-old male with hard nodular mass in right upper quadrant (shock symptoms reported)	Subcutaneous injection of two doses of the drug tyramin (0.06 g/dose). Second dose followed by i.v. dose of 6% Acacia in saline (500 cc)	Death accelerated by intravenous administration of Acacia solution	Lee 1922
Acacia	Male patient with pulmonary hemorrhage	Intravenous administration of 6% Acacia in saline (150 cc)	Patient's condition worsened immediately after injection, followed by death 2 h 20 min later	Lee 1922
Acacia	27-year-old female recovering from elephantiasis surgery	Intravenous administration of 6% Acacia solution (500 cc) and 500 cc of physiologic saline solution after initial surgery and after second operation 7 months later	No adverse effects after first infusion. Signs/symptoms noted after second infusion: nasal obstruction and lacrimation, followed by difficulty in breathing, coughing, and suggestion of laryngeal stridor. Symptoms disappeared rapidly after epinephrine administration	Maytum and Magath 1932
Acacia	15 kidney transplant patients. Itching/rash in 3 patients	Patients had been treated with prednisone and azathioprine for 10 months to 5 years. Prednisone tablets contained Acacia and tragacanth gums as adhesives. Itching/rash not observed after tablets withdrawn. Scratch tests performed	Scratch test results for 2 of 3 patients with reactions tested: Positive reactions to Acacia and tragacanth gums, respectively. Scratch test results negative in remaining transplant patients	Rubinger et al. 1978
Gum Arabic	65-year-old male with allergic reactions	Four allergic accidents experienced after drinking coffee. Gum arabic used to coat roasted coffee beans. Prick tests and human basophil degranulation tests performed	Dual sensitization to coffee and gum arabic	Moneret-Vautrin 1993
Gum Arabic	57-year-old male with chronic alveolitis	Chronic alveolitis due to repeated and prolonged inhalation of sweets containing gum arabic.	Progress satisfactory in terms of clinical status and lung function measurement after exposure discontinued	De Fenoyl et al. 1987

Acacia (crude and purified forms)	53-year-old plaster molder in candy factory with bronchial asthma	Bronchial asthma due to inhalation of dust from factory environment. Scratch and intradermal injection tests performed	Markedly positive reaction to crude Acacia. Purified Acacia more reactive; induced positive reactions when tested at concentrations as low as 1:5000 dilution in scratch and intradermal injection tests	Spielman and Baldwin 1933
Gum Arabic (extracted with sodium carbohydrate buffer)	50-year-old confectioner at candy factory with strong respiratory allergy to gum arabic	Skin prick and intracutaneous tests	No reaction to gum arabic (1:1000 dilution) in prick test. Intracutaneous test results: +++ (1:10,000 dilution), + (1:100,000 dilution), and (1:1,000,000 dilution). Evaluation of IgE antibody response indicated that patient's serum reacted strongly to gum arabic	Fötisch et al. 1998
Gum Arabic	53-year-old printer with asthma	Asthma due to exposure to offset spray containing gum arabic. Repeat cutaneous and intracutaneous tests performed	4+ reaction to gum arabic in repeat cutaneous and intracutaneous tests	Bohner et al. 1941
Gum Acacia	32 male printers with asthma	Exposure to spray (used in color-printing) containing Gum Acacia and isopropyl alcohol. Average duration of exposure = 4 to 8 years	Asthma developed after exposure to spray	Fowler 1952
Gum Arabic	12 employees of gum processing factory (office and mill workers)	Sensitization test performed	Seven of 12 workers had positive skin reactions to gum arabic. All 12 had respiratory symptoms that were of an allergic nature	Gelfand 1943
Gum Arabic (as supplied)	24-year-old printer with 3-month history of hand dermatitis	Exposure to gum arabic on the job. Patch tests (Finn chambers) performed	++ reaction to gum arabic	Freeman 1984
Wet clay containing 5 to 7% gum arabic	45-year-old female with rash on hands	Exposure to wet clay for 2 years on the job. Patch tests performed	+ reaction to 1% and 5% aqueous gum arabic. ++ reaction to 25% aqueous gum arabic	Ilchyshyn and Smith 1985
Gum Arabic	44-year-old lithoprinter with 2-year history of hand eczema	Exposure to gum arabic (used to coat printing plates) on the job. Eczema worsened after exposure to gum arabic. Patch testing of 10% aqueous gum arabic	Positive patch test reaction to 10% aqueous gum arabic	van Ketel 1984

sequence was repeated for a total of five induction exposures, after which a 10-day nontreatment period was observed. Prior to challenge patch application, a new site on the opposite arm, forearm, or side of the back was pretreated for 1 h with 5% aqueous SLS (0.1 ml under occlusive patch). A single challenge patch (occlusive patch) was then applied to the same site for 48 h. Reactions were scored at 1 and 24 h after patch removal according to the following scale: 0 (not sensitized) to 3 (strong sensitization [large vesiculobullous reaction]).

Sensitization reactions were not observed at 1 or 24 h after challenge patch removal. It was concluded that, under the conditions of this test, the mascara containing 8.0% Acacia Senegal did not possess a detectable contact-sensitizing potential, and, hence, is not likely to cause contact sensitivity reactions under normal use conditions (Ivy Laboratories 2000).

Acacia (Not Gum Arabic)

Letizia et al. (2000) evaluated the sensitization potential of Acacia Farnesiana Extract (from flowers, 4% in petrolatum) in a maximization test using 30 healthy male and female volunteers. The test substance was applied, under occlusion, to the same site on both forearms of each subject throughout induction. The induction phase consisted of a total of five 48-h exposures (on alternate days).

Prior to application of the initial induction patch, the test site was pretreated with 5% aqueous SLS, under occlusion. The induction phase was followed by a 14-day nontreatment period, after which a challenge patch was applied (48 h) to new sites on each subject. Challenge patch applications were preceded by 30 min applications of 2% aqueous SLS, under occlusion, on the left side. Challenge sites on the right side were not pretreated. A fifth challenge site (petrolatum applied) served as the control.

It was concluded that none of the reactions observed could be classified as a significant skin irritation or allergic reaction (Letizia et al. 2000).

Case Reports

Gum Arabic

Gelfand (1949) reported allergic disorders in 10 subjects (7 males, 3 females; 11 to 55 years old) who had ingested various gum-containing foods. Gum arabic was among the gums present in each food ingested. Some of the allergic symptoms reported included bronchial asthma, generalized urticaria, and vasomotor rhinitis. Allergic symptoms were not observed upon removal of suspect gum-containing foods from the diet, and symptoms were reproduced when clinical trials were repeated.

Positive skin reactions (test procedure not stated) to gum arabic were observed in each of the 10 subjects. The results of serologic studies (sera from four subjects) indicated that gum arabic was the dominant gum antigen in two subjects and that tragacanth and karaya were the dominant gum antigens in the remaining two subjects. The serological studies included passive transfer tests in serial dilutions and neutralization studies. It was determined that gum arabic and other vegetable gums could cause allergic disorders by ingestion in sensitive subjects (Gelfand 1949).

Raghuprasad et al. (1980) reported cross-reactivity between Gum Acacia and gum tragacanth in a 24-year-old patient who developed sensitization to Quillaja bark (*Quillaja saponaria*) dust, which resulted in rhinitis and asthma. The CIR Expert Panel has previously evaluated the safety of Tragacanth Gum in cosmetics, and concluded that this ingredient is safe in the present practices of use and concentration (Elder 1987). Specific immunoglobulin E(IgE) to pulverized Quillaja bark, gum arabic, and gum tragacanth were measured according to a modification of the radioallergosorbent test (RAST). Each of the three antigens (20 mg/ml) was coupled directly to methyl cellulose disks that had been activated previously by cyanogen bromide dissolved in acetonitrile. Results were expressed as percent binding.

The amount of radioactivity bound by the patient's serum was compared with control sera from healthy, nonallergic volunteers (number not stated) not known to be exposed to Quillaja bark dust. The mean percent binding of IgE to Quillaja bark in patient sera was 22.4%, compared to 3.2% for the control. Compared to negligible binding in control sera, significant binding was reported for gum arabic (32.5% binding) and gum tragacanth (30.8% binding) (Raghuprasad et al. 1980).

Additional case reports on gum arabic and other species of *Acacia* are summarized in Table 12. Although two fatalities are reported, neither related to gum arabic and most case reports involve sensitization reactions.

SUMMARY

This safety assessment includes the following ingredients, derived from Acacia, that are listed in the *International Cosmetic Ingredient Dictionary and Handbook*: Acacia Catechu Gum, Acacia Concinna Fruit Extract, Acacia Dealbata Leaf Extract, Acacia Dealbata Leaf Wax, Acacia Decurrens Extract, Acacia Farnesiana Extract, Acacia Farnesiana Flower Wax, Acacia Farnesiana Gum, Acacia Senegal Extract, Acacia Senegal Gum, and Acacia Senegal Gum Extract.

Gum arabic is another name for Acacia Senegal Gum. Gum arabic is generally recognized as safe for direct addition to food for human ingestion. Acacia Senegal Gum has been described as the major commercial Acacia gum. Gum arabic is produced when the Acacia tree is stressed by infection, poor nutrition, heat, or lack of moisture. The gum exudes through wounds in the bark that occur naturally or are purposely made to stimulate production.

Gum arabic is composed of D-galactose, L-rhamnose, L-arabinose, and D-glucuronic acid residues in an arrangement of a main chain of galactosyl units joined by β-D-($1 \rightarrow 3$) linkages and side chains or branched oligosaccharides linked to the main chain by β-D-($1 \rightarrow 6$) linkages. It has also been described as a complex mixture of calcium, magnesium, and potassium salts of arabic acid. Arabic acid is a complex of galactose, rhamnose, arabinose, and glucuronic acid.

Aflatoxin has been reported as an impurity in Acacia Catechu, but not in gum arabic. Acacia Gum (Acacia Senegal) did not contain detectable pesticide residues.

Qualitative information is available on the components that may be found in ingredients derived from various Acacia species and plant parts. This information indicates a few similarities and many differences in constituents. Quantitative or semiquantitative data were not available.

The principal UV absorbance of gum arabic occurred at wavelengths below 240 nm. At wavelengths above 240 nm, the UV absorbance was not significant.

Acacia Decurrens Extract and Acacia Farnesiana Extract are described as a cosmetic astringent, and Acacia Decurrens is also described as a skin-conditioning agent—occlusive, although none of these are reported to be in current use. Cosmetic functions of the other Acacia-derived ingredients included in this review are not described. Product formulation data submitted to the FDA indicated that Acacia was used in 22 cosmetic products—no information is available to further describe the species or plant part. Cosmetic use concentration data supplied by the cosmetics industry indicated maximum use concentrations of 9% in shampoos for Acacia Senegal Gum and 0.001% for Acacia Senegal Gum Extract, in bath soaps and detergents.

Recommended use concentrations of Acacia Concinna Fruit Extract are 1.0% to 2.0% for use in shampoos, hair packs, hair conditioners, and hair rinses, although no uses have been reported to FDA.

The weight gain for rats fed gum arabic at a dietary concentration of 16% was 75% of that reported for control rats. Approximately 80% of the gum arabic was absorbed. Results from other studies involving rats suggest that the metabolism of gum arabic is mediated by bacteria in the cecum.

Results of studies in which dogs and rabbits were injected intravenously with gum arabic indicated that gum arabic or some other product associated with it accumulated in the liver and remained in the tissues for several months. Nonlethal effects included disturbances in hemoglobin values, white blood cells, and serum proteins.

Based on absorption and metabolism studies an expert analysis determined that gum arabic is capable of being digested to simple sugars. It was also determined that conclusive evidence indicating that the intact gum arabic molecule is absorbed under normal conditions was lacking.

In an in vitro assay, dose-dependent uncoupling of oxidative phosphorylation was noted in groups of rats dosed orally with gum arabic up to 10% twice daily for 4 weeks, but comparable biochemical effects were not observed in vivo.

An acute oral LD_{50} of 8000 mg/kg was reported for Acacia Gum in rabbits. The acute oral LD_{50} for Acacia Farnesiana Extract (from flowers) in rabbits was >5.0 g/kg. A minimal lethal dose of >2 g/kg (10 ml/kg) for Acacia Dealbata Leaf Wax in a suspension with paraffin oil was reported in an acute oral toxicity study involving rats. None of the animals died, and no test substance-related lesions of organs examined were noted at necropsy. Similarly, in another study, no deaths or test substance-related, organ lesions were reported following the oral administration of Acacia Farnesiana Flower Wax at a dose of 10 ml/kg.

In an acute dermal toxicity study of Acacia Farnesiana Extract (from flowers) involving rabbits, an LD_{50} of >5.0 g/kg was reported.

Gum arabic did not cause any abnormal changes in serum chemistry parameters or induce toxicologically significant lesions in rats that received oral doses daily for 28 days. Gum arabic was also administered to rats in four other short-term oral toxicity studies. Collectively, test concentrations ranged from 1% to 20% and study durations ranged from 28 days to 9 weeks. No significant or discernible ultrastructural differences were found between tissues (heart, liver, small intestine) of control rats and test rats; hematological findings were normal. Gum arabic was nontoxic, even at the highest concentration tested.

One of three dogs injected intravenously (32 to 35 injections) with gum arabic over a period of 76 days died. The range for the total cumulative dose was 15.7 to 47.7 g/kg, and death occurred at the highest dose (47.7 g/kg). An enlarged liver was observed in the animal that died, and the cause of death was not determined. Enlarged livers and swollen kidneys were also observed in dogs that received doses ranging from 1 to 2 g/kg.

In a subchronic (13 weeks) oral toxicity study on Acacia Senegal Gum, the only treatment-related alteration noted in rats at necropsy was cecal enlargement in animals of the highest dose (14 g/kg/day) groups.

Electron microscopic findings for samples of livers and kidneys from groups of five rats fed diets containing 0.5% to 3.5% w/w Acacia Senegal Gum daily for 91 days were negative. Mitochondria and nuclei were ultrastructurally normal in appearance and internal structure.

The administration of a single dermal dose of Acacia Farnesiana Extract (from flowers, 5.0 g/kg) to rabbits induced moderate erythema and edema. Undiluted Acacia Dealbata Leaf Wax was classified as a non-irritant after application, under occlusive patches, to scarified skin of albino rabbits for 24 h. Undiluted Acacia Farnesiana Flower Wax was classified as a slight irritant when tested according to a similar procedure.

A 20.0% solution of Acacia Farnesiana Extract in methanol (from flowers) did not induce phototoxicity in SKH:hairless mice.

Anaphylactic signs in guinea pigs injected intraperitoneally (mild challenge reactions) or intravenously (strong challenge reactions) with Acacia solution have been reported. No signs of anaphylaxis were observed in rabbits injected intravenously (no challenge reaction) with Acacia solution. In rabbits and guinea pigs injected with 7% Gum Acacia solution, no deleterious effects on antibody production resulted.

Mouse footpad swelling test results indicated a significant increase in footpad thickness (compared to controls) in mice immunized by injection of gum arabic in saline and Freund's adjuvant. Antigen-specific hypersensitivity reactions were noted. In a similar test, footpad swelling was significantly suppressed (compared to controls) in mice dosed orally with gum arabic and then immunized by injection of gum arabic in saline and Freund's adjuvant. In another test, intradermal challenge after immunization of mice with Acacia Senegal Gum caused a significant increase in footpad thickness.

Gum arabic was not mutagenic in numerous in vitro mutagenicity tests using *Salmonella typhimurium, Saccharomyces cerevisiae,* and *Bacillus subtilis* bacterial strains. In an in vitro cytogenetics assay, though results were classified as slightly positive, gum arabic did not induce definite abnormal anaphase figures in diploid human embryonic lung (WI-38) fibroblasts. The mutagenicity of gum arabic was also evaluated in numerous in vivo assays, the results of which were mostly negative.

Statistically significant positive results were noted in one of the three dominant lethal tests (rat assay, but not in two mouse assays) that were performed. Further testing in the mouse heritable translocation test yielded negative results. In acute and short-term in vivo cytogenetics assays (rats), though no significant positive responses were observed, there may have been a slight positive response. It was stated that further tests and a detailed statistical evaluation are needed in order to confirm this possibility. There were no statistically significant findings in mouse chromosomal aberrations and sperm-head morphology assays. Negative results were also reported in micronucleus tests (mouse bone marrow smears) and other in vivo assays.

No evidence of carcinogenicity was observed in rats dosed intraperitoneally with gum arabic (1.75% or 7.0% in saline or water) three times per week for up to 15 weeks. In another study, tumors were not observed in guinea pigs injected intramediastinally with 0.1 ml of a gruel of gum arabic (single dose).

The carcinogenicity of gum arabic was also evaluated using 4-week-old F344 and 4- to 5-week-old $B6C3F_1$ mice. Low-dose animals were fed gum arabic at a concentration of 25 g/kg in the diet and high-dose animals were fed 50 g/kg for 103 weeks. Neoplasms were observed only in male rats, and were diagnosed as malignant lymphomas or leukemia-lymphoma. Compared to controls, no significant increases were observed in the incidence of either type of neoplasm at either of the two test concentrations; gum arabic was classified as noncarcinogenic in rats and mice.

Oral administration of gum arabic (1 ml) did not cause antifertility effects in female rats or the suppression of spermatogenesis in male rats. Gum arabic was not teratogenic when administered orally to mice at doses up to 1600 mg/kg. Oral doses of gum arabic up to 1600 mg/kg also were not teratogenic in rats and hamsters, and oral doses up to 800 mg/kg were not teratogenic in rabbits.

No effects on fertility or ovulation (4% gum arabic), or any abnormal variations in blastocysts (10% gum arabic) were found in rabbits. Gum arabic, at a concentration of 15%, failed to induce teratogenicity or other reproductive effects in female rats. Gum arabic (5%) also did not cause abnormal sperm development in hamsters. Embryotoxicity was not noted in mice injected intraperitoneally with a 1% aqueous suspension or mucilage prepared from gum arabic.

No evidence of absorption of intact gum arabic was found in 22 infants fed gum arabic in milk. In a patient with nephrosis, 20% of the gum arabic injected intravenously was excreted in the urine over a period of 6 weeks. Gum arabic was not detected in feces specimens collected from five male volunteers before or after administration of the gum.

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

In a 48-h closed patch test, Acacia Farnesiana Extract (from flowers, 4.0% in petrolatum) did not induce skin irritation in any of the 30 subjects tested. In an "in-use test," skin irritation was not observed in any of the 30 subjects tested with Acacia Concinna Fruit Extract (2% in natural base [such as carageenan] and a routine shampoo base). The test substance remained in contact with the scalp for 10 to 15 min, and skin irritation was evaluated immediately after application and 24 and 48 h later.

The skin sensitization potential of a mascara containing 8.0% Acacia Senegal was evaluated in the maximization test using 28 healthy adult volunteers. It was concluded that, under the conditions of this test, the mascara containing 8.0% Acacia Senegal did not possess a detectable contact-sensitizing potential, and, hence, is not likely to cause contact sensitivity reactions under normal use conditions.

The results of a study involving ten subjects who had ingested various gum-containing foods, indicated that gum arabic could cause allergic disorders in sensitive subjects. Analyses of sera from 4 of the 10 subjects indicated that gum arabic was the dominant gum antigen in two subjects. Cross-reactivity between gum arabic and gum tragacanth was reported for a 24-year-old patient who developed sensitization to Quillaja bark (*Quillaja saponaria*) dust, which led to rhinitis and asthma.

Neither significant skin irritation nor allergic reactions to 4% Acacia Farnesiana Extract (from flowers) in petrolatum were observed in a maximization test (30 subjects).

A number of case reports of gum arabic allergenicity have been identified in the published literature.

DISCUSSION

Extensive safety test data are available on gum arabic that demonstrate its safety in a wide variety of applications, including cosmetic use. Based on the available information, the Panel concluded that Acacia Senegal Gum is equivalent to gum arabic and should be considered safe as used in cosmetics. It also appears that gums from other species are not the same as Acacia Senegal Gum. It follows that the safety test data on gum arabic can be used to support the safety of Acacia Senegal Gum and not gum from other Acacia species. Because Acacia Senegal Gum Extract is derived from Acacia Senegal Gum, the Panel considered that Acacia Senegal Gum Extract would present no additional safety issues.

The Panel recognized the potential for allergic responses to gum arabic. However, because of negative results for all 25 subjects in a human maximization study (mascara containing 8%

Acacia Senegal) and the expected slow rate of dermal absorption of gum arabic due to its large molecular size and water solubility, the Panel determined that it is not likely that normal use of gum arabic in a cosmetic product would result in sensitization.

The Panel is concerned that the available data suggesting the absence of pesticide residues in Acacia plants harvested wild are limited. The Panel advised the industry that the total polychlorinated biphenyl (PCB)/pesticide contamination of any plant-derived cosmetic ingredient should be limited to not more than 40 ppm, with not more than 10 ppm for any specific residue. The Panel also advised that limits were appropriate for the following impurities: arsenic (3 mg/kg maximum), heavy metals (0.002% maximum), and lead (5 mg/kg maximum).

The limited safety test data on Acacia Farnesiana Extract and on Acacia Concinna Fruit Extract were not sufficient to assess the safety of these ingredients in cosmetics.

The Panel found no information that adequately characterized the composition of fruit, leaf or other extracts, leaf wax, flower wax, or gum from Acacia species other than *A. senegal*. Therefore, the Panel could not extrapolate the available data on gum arabic to support the safety of Acacia Catechu Gum, Acacia Concinna Fruit Extract, Acacia Dealbata Leaf Extract, Acacia Dealbata Leaf Wax, Acacia Decurrens Extract, Acacia Farnesiana Extract, Acacia Farnesiana Flower Wax, Acacia Farnesiana Gum, and Acacia Senegal Extract. The Panel concluded that available data are insufficient to support the safety of these Acacia-derived ingredients.

The additional data needed for these ingredients include

1. concentration of use in cosmetics;
2. identify the chemical composition; if they are sufficiently different from those of Acacia Senegal Gum, then the following data would be needed:
   a. UV absorption spectrum; if there is significant absorbance in the UVA or UVB range, then phototoxicity and photosensitization studies may be needed;
   b. with the exception of Acacia Farnesiana Extract and Acacia Concinna Fruit Extract, sensitization and irritation data are needed;
   c. two genotoxicity assays, one in a mammalian system; if positive, then a 2-year dermal carcinogenicity study using National Toxicology Program (NTP) methods may be needed;
   d. dermal absorption data; if there is any evidence of significant dermal absorption, then reproductive and developmental toxicity data may be needed.

CONCLUSION

Based on the available animal and clinical data included in this report, the CIR Expert Panel concluded that Acacia Senegal Gum and Acacia Senegal Gum Extract are safe as used in cosmetic products. The Panel also concluded that the available data are insufficient to support the safety of the following ingredients in cosmetic products: Acacia Catechu Gum, Acacia Concinna Fruit Extract, Acacia Dealbata Leaf Extract, Acacia Dealbata Leaf Wax, Acacia Decurrens Extract, Acacia Farnesiana Extract, Acacia Farnesiana Flower Wax, Acacia Farnesiana Gum, and Acacia Senegal Extract.

REFERENCES

Abdalla, M. H. 1988. Isolation of aflatoxin from *Acacia* and the incidence of *Aspergillus flavus* in the Sudan. *Mypathologia* 104:143–148.

Abul Gafar, M., T. Obata, F. Kiuchi, and Y. Tsuda. 1997. Acacia concinna saponins. I. Structures of prospogenols, concinnosides A-F, isolated from the alkaline hydrolysate of the highly polar saponin fraction. *Chem. Pharm. Bull. (Tokyo)* 45:620–625.

Akbarsha, M. A., and B. Manivannan. 1973. Biochemical changes in the testis and male accessory organs of albino rats on treatment with *Andrographis paniculata* (NEES). *Ind. J. Comp. Anim. Physiol.* 11:103–108.

Ames, B. N. 1971. The detection of chemical mutagens with enteric bacteria, pp. 267–282. In A. Hollaender (ed.), *Chemical Mutagens, Principles & Methods for Their Detection*. Plenum Press, New York, NY.

Ames, B. N., J. McCann, and E. Yamasaki. 1975. Methods of detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. *Mutat. Res.* 31:347–364.

Anderson, D. M. W. 1986. Evidence for the safety of gum arabic (*Acacia senegal (L.)* Wild.) as a food additive—a brief review. *Food Addit. Contam.* 3:225–230.

Anderson, D. M. W. 1988. The natural gums. *Bri. Nutr. Found. Nutr. Bull.* 13:101–113.

Anderson, D. M. W., P. Ashby, A. Busuttil, M. A. Eastwood, B. M. Hobson, A. H. Ross, and C. A. Street. 1982. Subchronic effects of gum arabic (*Acacia*) in the rat. *Toxicol. Lett.* 14:221–227.

Anderson, D. M. W., P. Ashby, A. Busuttil, S. A. Kempson, and M. E. Lawson. 1984. Transmission electron microscopy of heart and liver tissues from rats fed with gums arabic and tragacanth. *Toxicol. Lett.* 21:83–89.

Anderson, D. M. W., A. Busuttil, S. A. Kempson, and D. W. Penman. 1986. Transmission electron microscopy of jejunum, ileum, and cecum tissues from rats fed with gums arabic, karaya and tragacanth. *Toxicology* 41:75–82.

Anderson, D. M. W., D. M. B. Douglas, N. A. Morrison, and W. Weiping. 1990. Specifications for gum arabic (*Acacia senegal*); analytical data for samples collected between 1904 and 1989. *Food Addit. Contam.* 7:303–322.

Anderson, D. M. W., J. R. A. Millar, and W. Weiping. 1991. Gum arabic (*Acacia senegal*): Unambiguous identification by ^{13}C-NMR spectroscopy as an adjunct to the Revised JECFA Specification, and the application of ^{13}C-NMR spectra for regulatory/legislative purposes. *Food Addit. Contam.* 8:405–422.

Anonymous. 1993. Twenty Australian *Acacias* and the doctors whose lives and works they commemorate. *Med. J. Aust.* 159:731–738.

Aronson, S. B., and R. B. McMaster. 1972. Mechanism of the host response in the eye. VI. Immune and toxic stimulation of inflammation in the germ-free guinea pig. *Arch. Ophthalmol.* 88:533–539.

Avon Products, Inc. 2000a. UV absorption of *Acacia senegal*, Avon Code RI-0253. Analytical laboratory report (Analytical job number 69669). Unpublished data submitted by CTFA, March 27, 2000. (2 pages.)[2]

Avon Products, Inc. 2000b. UV absorption of acacia senegal, Avon Code RI-0253. Analytical laboratory report (Analytical job number 70384). Unpublished data submitted by CTFA, March 27, 2000. (2 pages.)[2]

Bachmann, E., E. Weber, M. Post, and G. Zbinden. 1978. Biochemical effects of gum arabic, gum tragacanth, methylcellulose, and carboxymethylcellulose-Na in rat heart and liver. *Pharmacology* 17:39–49.

Bertin Laboratories. 1987. Study of primary cutaneous tolerance of the product Cassia Essential Wax [Acacia Farnesiana Flower Wax] (Study No. F-950). English translation. Unpublished data submitted by CTFA, February 14, 2001. (11 pages.)[2]

[2]Available for review from the Director, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 310, Washington, DC 20036, USA

Biogir S. A. Conseil Recherche. 1990a. Assessment of acute toxicity in rat. Cire Essentielle De Fleurs De Mimosa [Acacia Dealbata Leaf Wax] (Study No. TAO 90.786). Unpublished data submitted by CTFA, February 14, 2001. (15 pages.)[2]

Biogir S. A. Conseil Recherche. 1990b. Assessment of cutaneous tolerance in rabbit. Index of primary cutaneous irritation. Cire Essentielle De Fleurs De Mimosa [Acacia Dealbata Leaf Wax] (Report No. IC 90.786). Unpublished data submitted by CTFA, February 14, 2001. (13 pages.)[2]

Bohner, C. B., J. M. Sheldon, and J. W. Trenis. 1941. Sensitivity to gum acacia, with a report of ten cases of asthma in printers. *J. Allerg.* 12:290–294.

Brusick, D. J., and V. W. Mayer. 1973. New developments in mutagenicity screening techniques using yeast. *Environ. Health Perspect.* 6:83–96.

Carlisle International Corporation. 1997a. Additional data for Acacia Concinna. First Submission. Unpublished data submitted by Carlisle International Corporation, September, 1997. (1 page.)[2]

Carlisle International Corporation. 1997b. Additional data for Acacia Concinna. Second Submission. Unpublished data submitted by Carlisle International Corporation, December, 1997. (6 pages.)[2]

Chaudhari, P. N., and V. G. Hatwalne. 1973. Effect of katha (*Acacia catechu*) on serum and liver cholesterol levels in rats. *Indian J. Nutr. Dietet.* 10:130–133.

Collins, T. F. X., J. J. Welsh, T. N. Black, S. L. Graham, and L. H. Brown. 1987. Study of the teratogenic potential of gum arabic. *Food Chem. Toxicolo.* 25:815–821.

Cook, W. M., R. Purchase, G. P. Ford, D. M. Creasy, P. G. Brantom, and D. Gangolli. 1992. A 28-day feeding study with ethyl acetoacetate in rats. *Food Chem. Toxicolo.* 30:567–573.

CTFA. 2000a. Use data collected by CTFA in 1999. Unpublished data submitted by CTFA. (1 page.)[2]

CTFA. 2000b. Unpublished data submitted by CTFA. (1 page.)[2]

Dangerous Properties of Industrial Materials Report. 1981. Hazardous Materials Section. *Dangerous Properties of Industrial Materials Report* 1:17–87.

De Fenoyl, O., F. Capron, F. Guyon, B. Lebeau, and J. Rochemaure. 1987. Inhalation pneumonia presenting as a pneumocystitis infection. *Rev. Mal. Respir.* 4:97–100.

Elder, R. L., ed. 1987. Final report on the safety assessment of tragacanth gum. *J. Am. Coll. Toxicol.* 6:1–22.

El-Hamid, A., and I. Sidrak. 1970. The investigation of *Acacia farnesiana* essential oil. *Planta Med.* 18:98–100.

European Economic Community (EEC). 2001. EEC Cosmetics Directive 76/768/EEC, as amended, Annexes I through VII. Brussels: EEC.

European Federation for Cosmetic Ingredients (EF*f*CI). 2000. Unpublished data on Acacia catechu, Acacia concinna, Acacia decurrens var. dealbata, Acacia decurrens, Acacia farnesiana, Acacia arabica=Acacia nilotica, Acacia mearnsii, Acacia melanoxylon, Acacia nilotica, Acacia senegal, Arabic gum, and Acacia ssp. Submitted by the EF*f*CI.[2]

Federation of American Societies for Experimental Biology (FASEB). 1973. Evaluation of the health aspects of gum arabic as a food ingredient. NTIS Report No. PB-234 904.

Food and Agriculture Organization of the United Nations (FAO). 1990. Specifications for identity and purity. *Food Nutr. Paper* 49:23–25.

Food and Agriculture Organization of the United Nations (FAO). 1999. *Compendium of food additive specifications. Addendum 7* (Joint FAO/World Health Organization Expert Committee on Food Additives. 53rd Session), 1–2 Rome: FAO.

Food Chemicals Codex. 1996. Specifications on acacia. *Food Chemicals Codex*, 4th ed., 9. Washington, DC: National Academy Press.

Food and Drug Administration (FDA). 2001. Frequency of use of cosmetic ingredients. *FDA Database*. Washington, DC: FDA.

Food and Drug Research Laboratories. 1972. Teratologic evaluation of FDA 71–75 (Gum Arabic). NTIS Report No. PB-221 796.

Fötisch, K., J. Fäh, B. Wüthrich, F. Altmann, D. Haustein, and S. Vieths. 1998. IgE antibodies specific for carbohydrates in a patient allergic to gum arabic (*Acacia senegal*). *Allergy* 53:1043–1051.

Fowler, P. B. S. 1952. Printer's asthma. *Lancet* 2:755–757.

Freeman, S. 1984. Allergic contact dermatitis due to 1,2-benzisothiazolin-3-one in gum arabic. *Contact Dermatitis* 11:146–149.

Frohberg, H., H. Oettel, and H. Zeller. 1969. Mechanism of the teratogenic effect of tragacanth. *Arch. Toxicol.* 25:268–295.

Frutarom Meer Corporation. No date. Product information on gum arabic. Unpublished data submitted by CTFA, March 27, 2000. (2 pages.)[2]

Gabridge, M. G., and M. S. Legator. 1969. A host-mediated microbial assay for the detection of mutagenic compounds. *Proc. Soc. Exp. Biol. Med.* 130:831.

Gelfand, H. H. 1943. The allergenic properties of the vegetable gums. *J. Allergy* 14:203–219.

Gelfand, H. H. 1949. The vegetable gums by ingestion in the etiology of allergic disorders. *J. Allergy* 20:311–321.

Gennaro, A. R., ed. 1990. *Remington's Pharmaceutical Sciences*, 18th ed., 1304. Easton, PA: Mack Publishing Company.

Gottschalck, T. A., and G. N. McEwen, Jr., eds. 2000. *International Cosmetic Ingredient Dictionary and Handbook*, 10th ed. Washington, DC: CTFA.

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

Hagos, M., and G. Samuelsson. 1988. Quantitative determination of quracol A, B and (+)-fisetinidol in bark and gum of *Acacia tortilis*. *Acta Pharm. Suec.* 25:321–324.

Haworth, S., T. Lawlor, K. Mortelmans, W. Speck, and E. Zeiger. 1983. *Salmonella* mutagenicity results for 250 chemicals. *Environ. Mutagen* 5:3–142.

Heidelberger, M., O. T. Avery, and W. F. Goebel. 1929. A soluble specific substance derived from gum arabic. *J. Exp. Med.* 49:847–857.

Huynh, P. N., A. P. S. Hikim, C. Wang, K. Stefonovic, Y. H. Lue, A. Leung, V. Atienza, S. Baravarian, V. Reutrakul, and R. S. Swerdloff. 2000. Long-term effects of triptolide on spermatogenesis, epididymal sperm function, and fertility in male rats. *J. Androl.* 21:689–699.

Ilchyshyn, A., and G. Smith. 1985. Gum arabic sensitivity associated with epidemic hysteria dermatological. *Contact Dermatitis* 13:282–283.

Informatics Inc. 1972. GRAS (Generally Recognized as Safe) food ingredients—gum arabic. NTIS Report No. PB 223 614.

Ishizaki, M., and S. Ueno. 1987. The DNA-damaging activity of natural food additives (IV). *Shokuhin Eiseugaku Zasshi (J. Food Hyg. Soc. Japan)* 28:498–501.

Ivy Laboratories. 2000. An evaluation of the contact-sensitization potential of a topical coded product (mascara containing 8% Acacia Senegal) in human skin by means of the maximization assay. Unpublished data submitted by CTFA, March 27, 2000. (11 pages.)[2]

Jain, A. K., K. Shimoi, Y. Nakamura, I. Tomita, and T. Kada. 1987. Preliminary study on the desmutagenic and antimutagenic effect of some natural products. *Curr. Sci.* 57:1266–1269.

Joint FAO/WHO Expert Committee on Food Additives (JECFA). 1990. Evaluation of certain food additives & contaminants. 35th Report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, No. 789.

Joshi, K. C., M. K. Tholia, and T. Sharma. 1975. Chemical examination of *Acacia modesta*. *Planta Med.* 27:281–283.

Kar, R. N., K. Khan, and S. K. Mukherjee. 1984 *In vivo* mutagenic effect of methyldopa. 1. Dominant lethal test in male mice. *Cytobios* 41:163–164.

Katz, M., J. A. Heddle, and M. F. Salamone. 1981. Mutagenic activity of polycyclic aromatic hydrocarbons and other environmental pollutants. In: a *Polynuclear Aromatic Hydrocarbons: Chemical Analysis and Biological Fate*, ed. M. Cooke and A. J. Dennis, 519–528. Columbus, OH: Battelle Press.

Lawrence, J. F., and J. R. Iyengar. 1985. Gas chromatographic determination of polysaccharide gums in foods after hydrolysis and derivatization. *J. Chromatogr.* 350:237–244.

Lee, R. V. A. 1922. Sudden death in two patients following intravenous injections of acacia. *JAMA* 26:726–727.

Letizia C. S., J. Cocchiara, G. A. Wellington, C. Funk, and A. M. Api. 2000. Monographs on Fragrance Raw Materials. Cassie Absolute (Acacia Farnesiana (L.) Willd.). *Food Chem. Toxicol.* 38:S27–S29.

Lewis, R. J., ed. 1993a. *Hawley's Condensed Chemical Dictionary*, 12th ed., 93. New York: Van Nostrand Reinhold.

Lewis, R. J., ed. 1993b. *Hazardous Chemicals Desk Reference*, 3rd ed., 87. New York: Van Nostrand Reinhold.

Litton Bionetics, Inc. 1975. Mutagenic evaluation of compound. FDA 71-15 PM9000-01-5, gum arabic. NTIS Report No. PB-267 348.

Lutz, W. K., E. Brandle, and G. Zbinden. 1978. Effect of gum arabic on aminopyrine demethylation in rats. *Experentia* 34:1609–1610.

MacGregor, J. T., C. M. Wehr, G. D. Manners, L. Jurd, J. L. Minkler, and A. V. Carrano. 1983. *In vivo* exposure to plant flavonols. Influence on frequencies of micronuclei in mouse erythrocytes and sister-chromatid exchange in rabbit erythrocytes. *Mutat. Res.* 124:255–270.

Marakesh, I., Y. Y. Mei, M. Go, and J. Haginawa. 1969. Isolation of (−)-trans-4-hydroxypipecolic acid from the young leaves of *Acacia mollissima*. *Yakugaku Zasshi* 89:1723–1725.

Marrack, J., and B. R. Carpenter. 1938. The cross reactions of vegetable gums with type II antipneumococcal serum. *Br. J. Exp. Pathol.* 19:53–65.

Matsuzawa, S. 1968. Immunochemical studies on gum arabic. 4) Blood group antigens other than Le^a in gum arabic. *Nippon Hoigaku Zasshi* 22:283–289.

Maxwell, W. A., and G. W. Newell. 1974. Screening techniques for environmental mutagens. In *Molecular & Environmental Aspects of Mutagenesis*, 6th Rochester International Conference on Environmental Toxicity (1973). Louise Prakash, ed. Ch 14, pp. 223–252.

Maytum, C. K., and T. B. Magath. 1932. Sensitivity to acacia. *JAMA* 99:2251.

Melnick, R. L., J. Huff, J. K. Haseman, M. P. Dieter, C. K. Grieshaber, D. S. Wy, A. B. Russfield, A. S. K. Murthy, R. W. Fleischman, and H. S. Lilja. 1983. Chronic effects of agar, guar gum, gum arabic, locust-bean gum, or tara gum. *Food Chem. Toxicol.* 21:305–311.

Ministry of Health, Labor and Welfare (MHLW). 2000a. Unofficial translation of MHW Ordinance No. 331, Attached Table 1 [Negative List]. Ministry of Health Labor and Welfare, Pharmaceutical and Medical Safety Bureau, Inspection and Guidance Division, 2-2,1-chome, Kasumigaseki, Chiyoda-ku, Tokyo 100-8045, Japan.

MHLW. 2000b. Unofficial translation of MHW Ordinance No. 331, Attached Table 2 [Restricted List]. Ministry of Health Labor and Welfare, Pharmaceutical and Medical Safety Bureau, Inspection and Guidance Division, 2-2,1-chome, Kasumigaseki, Chiyoda-ku, Tokyo 100-8045, Japan.

Moneret-Vautrin, D. A., G. Kanny, J. P. Faller, D. Levan, and C. Kohler. 1993. Severe anaphylactic shock with heart arrest caused by coffee and gum arabic, potentiated by beta-blocking eyedrops. *Rev. Med. Interne* 14:107–111.

Morseth, S. L., and T. Ihara. 1989a. Teratology study in rats with manidipine hydrochloride. *Yakuri To Chiryo* 17:163–183.

Morseth, S. L., and T. Ihara. 1989b. Reproduction study in rats with manidipine chloride. *Yakuri To Chiryo* 17:145–162.

Narita, K. 1985. Immunogenic specificity of gum arabic and genetic regulation of $Lewis^a$ expression. *Nippon Hoigaku Zasshi* 39:275–290.

Neal, S. B., and G. S. Probst. 1983. Chemically-induced sister-chromatid exchange in vivo in bone marrow of Chinese hamsters. An evaluation of 24 compounds. *Mutat. Res.* 113:33–43.

Nichols, W. W., P. Moorhead, and G. Brewen, 1971. Chromosome methodologies in mutation testing. *Newslett. Environ. Mutagen Soc.* 5:20.

Oelrichs, P. B., and T. McEwan. 1962. The toxic principle of *Acacia georginae*. *Queensland J. Agr. Sci.* 19:1–16.

Ojha, D., G. Singh, and Y. N. Upadhyaya. 1969. Clinical evaluation of *Acacia catechu*, Wild (Khadira) in the treatment of lepromatous leprosy. *Int. J. Lepr. Other Mycobat. Dis.* 37:302–307.

Osswald, H. 1968. Influence of various polysaccharides on the growth behavior of the Ehrlich ascites tumor. *Arzneimittelforschung* 18:1495–1498.

Parton, J. W., G. S. Probst, and M. L. Garriott. 1988. The *in vivo* effect of 2,6-xylidine on induction of micronuclei in mouse bone marrow cells. *Mutat. Res.* 206:281–283.

Parton, J. W., J. E. Beyers, M. L. Garriott, and R. N. Tamura. 1990. The evaluation of a multiple dosing protocol for the mouse bone-marrow micronucleus assay using benzidine and 2,6-xylidine. *Mutat. Res.* 234:165–168.

Partridge, S. M., and W. T. J. Morgan. 1942. Artificial antigens with agar, gum acacia, and cherry gum specificity. *Br. J. Exp. Pathol.* 23:84–94.

Pazur, J. H., S. A. Kelly-Delcourt, F. J. Miskiel, L. Burdett, and J. J. Docherty. 1986. The isolation of anti-gum arabic antibodies by affinity chromatography. *J. Immunol. Methods* 89:19–25.

Pentiah, P. R., P. P. Reddy, and O. S. Reddi. 1989. Induction of micronuclei in bone marrow cells of mice treated with phenylbutazone. *Indian J. Exp. Biol.* 18:869–871.

Prasad, M. H., K. Pushpavathi, P. Rita, and P. P. Reddy. 1987. The effect of thiram on the germ cells of male mice. *Food Chem. Toxicol.* 25:709–710.

Prival, M. J., V. F. Simmon, and K. E. Mortelmans. 1991. Bacterial mutagenicity testing of 49 food ingredients gives very few positive results. *Mutat. Res.* 26:321–329.

Quereshi, M. Y., D. J. Pilbeam, C. S. Evans, and A. Bell. 1977. The neurolathyrogen, α-amino-β-oxalylaminopropionic acid in legume seeds. *Phytochemistry* 16:477–480.

Raghuprasad, M. D., S. M. Brooks, A. Litwin, J. J. Edwards, I. L. Bernstein, and J. Gallagher. 1980. Quillaja bark (soapbark)-induced asthma. *J. Allergy Clin. Immunol.* 65:285–287.

Rice, C. E. 1954a. Relative effects of various agents on complement and antibody production. *J. Immunol.* 73:375–382.

Rice, C. E. 1954b. An investigation of some of the factors determining the decrease in complement activity in anaphylactic shock. *J. Immunol.* 75:85–95.

Ross, A. H. M., M. A. Eastwood, J. R. Anderson, and D. M. W. Anderson, 1984a. A study of the effect of dietary gum arabic in humans. *Am J. Clin. Nutr.* 37:368–375.

Ross, A. H. M., M. A. Eastwood, W. G. Brydon, A. Busuttil, L. F. McKay, and D. M. W. Anderson. 1984b. A study of the effects of dietary effects of gum arabic in the rat. *Br. J. Nutr.* 51:47–56.

Roy, A. K., and V. Kumari. 1991. Aflatoxin and citrinin in seeds of some medicinal plants under storage. *Int. J. Pharmacogn* 29:62–65.

Roy, A. K., K. K. Sinha, and H. K. Chourasia, 1988. Aflatoxin contamination of some common drug plants. *Appl. Environ. Microbiol.* 54:842–843.

Rubinger, D., M. Friedlander, and E. Superstine. 1978. Hypersensitivity to tablet additives in transplant recipients on prednisone. *Lancet* 2:689.

Sabir, M., and M. K. Razdan. 1970. Antifertility study with leaf extract of *Adina cordifolia* (Karam Ki Gaach). *Indian J. Physiol. Pharmacol.* 14:209–210.

Schardein, J. L., T. Woosley, L. E. Hamilton, and D. H. Kaump. 1965. Effects of aspirin and phenylbutazone on the rabbit blastocyst. *J. Reprod. Fertil.* 10:129–132.

Secor, J. B., E. E. Conn, J. E. Dunn, and D. S. Seigler. 1976. Detection and identification of cyanogenic glucosides in six species of Acacia. *Phytochemistry* 15:1703–1706.

Seigler, D. S., J. E. Dunn, E. E. Conn, and J. F. Pereira. 1983. Cyanogenic glycosides from four Latin American species of *Acacia. Biochem. Syst. Ecol.* 11:15–16.

Sethi, N., R. K. Singh, and R. K. Srivastava. 1989. Embryotoxicity of synthetic steroid RU 38486. *Biol. Mem.* 15:107–110.

Shaligram, S. G., and J. R. Vakil. 1990. Report on dermocosmetological study by in-use testing of Folicon 100: Shikakai Extract (Acacia Concinna) in cosmetic formulations. Unpublished data submitted by CTFA, October 19, 2000. (9 pages.)[2]

Sham, J. S., K. W. Chiu, and P. K. T. Pang. 1984. Hypotensive action of *Acacia catechu*. *Planta Med.* 50:177–180.

Sheu, C. W., K. T. Cain, C. J. Rushbrook, T. A. Jorgenson, and W. M. Generoso. 1986. Tests for mutagenic effects of ammoniated glycyrrhizin, butylated hydroxytoluene, and gum arabic in rodent germ cells. *Environ. Mutagen* 8:357–368.

Silvette, H., O. Swineford, and L. Tull. 1955. Observations on acacia as an immunizing, sensitizing anaphylactogenic and desensitizing antigen. *J. Allergy* 26:509–518.

Smith, C. J., P. A. Williams, M. Jones, and G. O. Phillips. 1990. Procedure for detection of aflatoxins in gum arabic samples. *Food Hydrocolloids* 4:221–225.

Societe Bertin. 1987. Research on the acute toxicity in rats (using the oral method) of the product: Cire Essentielle Cassie [Acacia Farnesiana Flower Wax] (Study No. F 949/5304). English translation. Unpublished data submitted by CTFA, February 14, 2001. (16 pages.)[2]

Sotohy, S. A., W. Muller, and A. A. Ismail. 1995. "In vitro" effect of Egyptian tannin-containing plants and their extracts on the survival of pathogenic bacteria. *Dtsch. Tierarztl. Wochenschr*. 102:344–348.

Spielman, A. D., and H. S. Baldwin. 1933. Atopy to acacia (gum arabic). *JAMA* 101:444–445.

SRI International. 1980. Microbial mutagenesis testing of substances. Compound report: F76-069, gum arabic, Stein-Hall Co., Lot No. 55-3701—Final Report. NTIS Report No. PB89 178636/AS.

Strobel, S., and A. Ferguson. 1986. Induction of oral tolerance, in mice, to gum arabic. *Food Addit. Contam.* 3:43–46.

Strobel, S., A. Ferguson, and M. W. Anderson. 1982. Immunogenicity of foods and food additives—*In vivo* testing of gums arabic, karaya, and tragacanth. *Toxicol. Lett.* 14:247–252.

Strobel, S., A. Ferguson, and D. M. W. Anderson. 1986. Immunogenicity, immunological cross reactivity and non-specific irritant properties of the exudate gums, arabic, karaya and tragacanth. *Food Addit. Contam.* 3:47–56.

Tezuka, Y., K. Honda, A. H. Banskota, M. M. That, and S. Kadota. 2000. Kinmoonosides A-C, three new cytotoxic saponins from the fruits of *Acacia concinna*, a medicinal plant collected in Mayanmar. *J. Nat. Prod.* 63:1658–1664.

Tlolka-Pluszczyk, J. 1970. Induction of neoplasms in guinea pigs by intramediastinal administration of methylcholanthrene. *Pol. Med. J.* 10:1186–1200.

Trivedi, C. P., N. T. Modi, R. K. Sarin, and S. S. Rao. 1986. Bronchodilator and anti-inflammatory effect of glycosidal fraction of *Acacia farnesiana. Indian J. Physiol. Pharmacol.* 30:267–268.

United States Pharmacopeial Convention, Inc. 2000. National Formulary Specifications on Acacia. *National Formulary*, 2209. Rockville, MD: United States Pharmacopeial Convention, Inc.

van Ketel, W. G. 1984. Simultaneous sensitization to gum arabic and cobalt. *Contact Dermatitis* 10:180.

Vogel, H. H., and R. Zaldivar. 1971. Carcinogenesis: The interaction of chemical and physical agents. *Radiat. Res.* 47:644–659.

Waller, D. P., N. Bunyapraphatsara, A. Martin, C. J. Vournazos, M. S. Ahmed, D. D. Soejarto, G. A. Cordell, H. H. S. Fong, L. D. Russel, and J. P. Malone. 1983. Effect of (+)-gossypol on fertility in male hamsters. *J. Androl.* 4:276–279.

Wassel, G. M., S. M. Abd El-Wahab, E. A. Aboutabl, N. M. Ammar, and M. S. Afifi. 1992. Phytochemical examination and biological studies of *Acacia nilotica L. Wild and Acacia farnesiana L. Wild* growing in Egypt. *Egypt J. Pharm. Sci.* 33:327–340.

Wenninger, J. A., and G. N. McEwen, Jr., eds. 1995. *International Cosmetic Ingredient Dictionary*, 6th ed., Vol 1, 1–2. Washington, DC: CTFA.

Wenninger J. A, G. N. McEwen, Jr., eds. 1997. *International Cosmetic Ingredient Dictionary and Handbook*., 7th ed., Vol 1, 2–3. Washington, DC:CTFA.

West Coast Analytical Service, Inc. 1999. Laboratory report on Acacia gum (Lot AN-1292). Analysis: Selected pesticides by GC/ECD and GC/TSD. Unpublished data submitted by CTFA, July 31, 2000. (7 pages.)[2]

Wild, D., E. Gocke, D. Harnasch, G. Kaiser, and M-T. King. 1985. Differential mutagenic activity of IQ (2-amino-3-methylimidazo[4,5-f]quinoline) in *Salmonella typhimurium* strains *in vitro* and *in vivo*, in *Drosophila* and in mice. *Mutat. Res.* 156:93–102.

Winter, C. A., E. A. Riseley, and G. W. Nuss. 1962. Carrageenin-induced edema in hind paw of rat as an assay for anti-inflammatory drugs. *Proc. Soc. Exp. Biol. Med.* 3:544–547.

World Health Organization (WHO). 1974. *Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers, and thickening agents*, 316–319. Geneva: WHO.

World Health Organization (WHO). 1990. Gum arabic. *WHO Food Additives Series, 26. Toxicological evaluation of certain food additives and contaminants*, 35th ed., 77–79. Geneva: WHO.

Yegnanarayan, R., and G. V. Joglekar. 1978. Anti-fertility effect of non-steroidal anti-inflammatory drugs. *Japan J. Pharmacol.* 28:909–917.

Zeiger, E., B. Anderson, S. Haworth, T. Lawlor, and K. Mortelmans. 1992. *Salmonella* mutagenicity tests: V. Results from the testing of 311 chemicals. *Environ. Mol. Mutagen.* 19:2–141.