Final Report on the Safety Assessment of PEG-6, -8, and -20 Sorbitan Beeswax[1]

Polyethylene Glycol (PEG)-6, -8, and -20 Sorbitan Beeswax are ethoxylated derivatives of Beeswax that function as surfactants in cosmetic formulations. Only PEG-20 Sorbitan Beeswax is currently reported to be used, at concentrations up to 11%. Few data on the PEGs Sorbitan Beeswax ingredients were available. This safety assessment relied upon the available data from previous safety assessments of Beeswax, Synthetic Beeswax, Sorbitan Esters, PEGs, and PEG Sorbitan fatty acid esters, also known as Polysorbates. The ester linkage of PEG Sorbitan fatty acid esters was hydrolyzed after oral administration, and the PEG Sorbitan moiety was poorly absorbed from the gastrointestinal tract. Sorbitan Stearate was hydrolyzed to stearic acid and anhydrides of sorbitol in the rat. PEGs are readily absorbed through damaged skin and are associated with contact dermatitis and systemic toxicity in burn patients. PEGs were not sensitizing to normal skin. PEGs did not cause reproductive toxicity, nor were tested PEGs mutagenic or carcinogenic. Sorbitol was not a reproductive or developmental toxin in multigenerational studies in rats. Neither Beeswax nor Synthetic Beeswax produced significant acute animal toxicity, ocular irritation, skin irritation, or skin sensitization. Polysorbates produced no acute or long-term effects, were generally not irritating or sensitizing, and were noncarcinogenic, although studies did demonstrate enhancement of the activity of chemical carcinogens. Sorbitan fatty acid esters were relatively nontoxic via ingestion, generally were not skin irritants or sensitizers, and were not mutagenic or carcinogenic. Sorbitan Laurate was a cocarcinogen in a mouse skin-painting study. PEG-6 Sorbitan Beeswax delivered via a stomach tube was nontoxic in rats in acute studies. Undiluted PEG-6 Sorbitan Beeswax was nonirritating to the eyes of rabbits and was nonirritating to intact and abraded skin of rabbits. PEG-20 Sorbitan Beeswax was only minimally irritating to rabbit eyes at concentrations as high as 30%, and was not a significant skin irritant in rabbits exposed to a product with PEG-20 Sorbitan Beeswax at 2%. In clinical tests, PEG-6 and -20 Sorbitan Beeswax at concentrations up to 3% were only minimally irritating and were nonsensitizers. Careful consideration was made of the data on the cocarcinogenesis, but the high exposure levels, high frequency of exposure, and absence of a dose-response led to the conclusion that there was not a cocarcinogenesis risk with the use of these ingredients in cosmetic formulations. Accordingly, these ingredients were considered safe for use in cosmetic formulations under the present practices of use.

Received 19 September 2001; accepted 11 October 2001.

[1]Reviewed by the Cosmetic Ingredient Review Expert Panel. Rebecca S. Lanigan and Torill A. Yamarik, former Scientific Analysts and Writers, prepared this report. Address correspondence to Dr. F. Alan Andersen, Director, Cosmetic Ingredient Review 1101 17th Street, NW, Suite 310, Washington, DC 20036, USA.

INTRODUCTION

Polyethylene Glycol (PEG)-6, -8, and -20 Sorbitan Beeswax are the ethoxylated derivatives of Beeswax that function as surfactant—emulsifying agents and surfactant—solubilizing agents in cosmetic formulations.

The Cosmetic Ingredient Review (CIR) Expert Panel has previously reviewed the safety in cosmetics of PEGs, Polysorbates (PEGs Sorbitan Fatty Acid Ester), Beeswax, Synthetic Beeswax, and Sorbitan Fatty Acid Esters. The conclusions reached in those reviews are described below:

- PEG-6, -8, -32, -75, -150, -14M, and -20M are safe for use at the concentrations reflected in the Cosmetic Use section and in the product formulation safety test data included in this report. The Expert Panel recommends that cosmetic formulations containing these PEGs not be used on damaged skin (Andersen 1993).
- Polysorbates 20, 21, 40, 60, 61, 65, 80, 81, and 85 (PEGs Sorbitan Fatty Acid Esters) are safe as cosmetic ingredients in the concentration of present use (Elder 1984a).
- Candelilla Wax, Carnauba Wax, Japan Wax, and Beeswax are safe as used in cosmetics under present practices of concentration and use (Elder 1984b).
- Ozokerite, Ceresis, Montan Wax, Paraffin, Microcrystalline Wax, Emulsifying Wax N.F., Synthetic Wax, and Synthetic Beeswax are safe as cosmetic ingredients in present practices of concentration and use (Elder 1984b).
- Sorbitan Stearate, Sorbitan Laurate, Sorbitan Sesquioleate, Sorbitan Oleate, Sorbitan Tristearate, Sorbitan Palmitate, and Sorbitan Trioleate (Sorbitan Fatty Acid Esters) are considered safe as cosmetic ingredients under present conditions of concentration and use (Elder 1985).
- Sorbitan Caprylate, Sorbitan Cocoate, Sorbitan Diisostearate, Sorbitan Dioleate, Sorbitan Distearate, Sorbitan Isostearate, Sorbitan Olivate, Sorbitan Sesquiisostearate, Sorbitan Sesquistearate, and Sorbitan Triisostearate (Sorbitan Fatty Acid Esters) are considered safe for use in cosmetic formulations under the present practices of use (CIR 1999).

International Journal of Toxicology, 20(Suppl. 4):27–38, 2001

1091-5818/01 $12.00 + .00

Because few data on the PEGs Sorbitan Beeswax were available, selected data on the PEGs, Polysorbates, the Beeswaxes, and the Sorbitan Fatty Acid Esters from these safety assessments, as well as new safety test data available since the reports were written, have been added to this report as a further basis to the assessment of safety in cosmetics of the PEGs Sorbitan Beeswax ingredients.

CHEMISTRY

Definition and Structure

PEGs Sorbitan Beeswax

The PEGs Sorbitan Beeswax (CAS Nos. 8051-15-8 and 8051-73-8) are ethoxylated sorbitan derivatives of Beeswax with an average of *n* moles of ethylene oxide, where *n* equals the number in the name. Synonyms for PEG-6, -8, and -20 Sorbitan Beeswax are Polyethylene Glycol *n* Sorbitan Beeswax, where *n* equals 300, 400, and 1000, respectively. These ingredients are also known as Polyoxyethylene (*n*) Sorbitan Beeswax (Wenninger and McEwen 1997).

The definition and structure of most of the related ingredients are presented in the safety assessments described above and will not be repeated here. Because Beeswax is the unusual component in these ingredients, information from previous reports is summarized below.

Beeswax

Beeswax is a complex mixture of several chemical entities, each with its own chemical and physical properties. Beeswax is synthesized from even-numbered alcohols ranging from C14 to C32. The alcohols are oxidized and combined with higher alcohols to form esters. Mixed dimers can be formed by the combination of certain acids and hydrocarbons by decarboxylation of esters. Beeswax contains 14% hydrocarbons, 73% esters (35% monoesters, 14% diesters, 3% triesters, 4% hydroxymonoesters, 8% hydroxypolyesters, 2% acid monoesters, and 7% acid polyesters), 12% free myristic acid, and unreported amounts of hydroxy acids and diols (Elder 1984b). Synthetic Beeswax is a blend of fatty esters (C32 to C62), fatty acids (C16 to C36), fatty alcohols (C16 to C36), and high-molecular-weight hydrocarbons (C21 to C34). Esters are the most abundant, the hydrocarbons next, the acids, and then the alcohols (Elder 1984c).

Chemical and Physical Properties

PEGs Sorbitan Beeswax

PEG-6 Sorbitan Beeswax is a tan, waxy solid with a "fatty odor." It is soluble in corn oil, but is insoluble in ethylene glycol, mineral oil, or water. The pour point is approximately 62°C. The hydroxyl number is 105 to 135, the saponification number is 65 to 90, and the maximum acid number is 4.0. PEG-6 Sorbitan Beeswax contains up to 1.5% moisture.

PEG-20 Sorbitan Beeswax is a tan, waxy solid with a mild fatty odor. It is soluble in warm corn oil, insoluble in water or ethanol, and dispersible in mineral oil. The pour point is approximately 63°C, the maximum acid number is 3.0, the maximum amount of moisture is 3.0%, and the saponification number is 70 to 105 (Nikitakis and McEwen 1990).

Impurities

PEGs Sorbitan Beeswax

Impurities data were not available on the PEGs Sorbitan Beeswax ingredients.

PEGs

PEG-6 could contain small amounts of monomer and dimers. The amounts have not been quantified. Peroxides, formed as a result of autoxidation, were found in PEG-32 and PEG-75. The amount of peroxide in PEG was dependent upon the molecular weight of the PEG and its age. The older the compound, the greater the concentration of peroxides. In a colorimetric assay used to determine the peroxide concentrations in several production lots of PEG, PEG-6 and PEG-8 were each added to acidified potassium iodide solution, and the iodine liberated was titrated against a standard thiosulfate solution. PEG-6 had peroxide concentrations ranging from 1.4 to 9.3 μEq thiosulfate/ml glycol. PEG-8 had concentrations ranging from 3.24 to 5.7 μ Eq thiosulfate/ml glycol. The specific peroxides present in the PEGs were not determined, but they were considered organic peroxides rather than hydrogen peroxide (Andersen 1993).

Beeswax

Natural impurities found in Beeswax include resins, pollens, and insect and plant matter, all of which are removed in the refining process. Refined Beeswax can contain additives such as tallow, paraffin, ceresin, and vegetable waxes (Elder 1984b).

Sorbitan Fatty Acid Esters

Sorbitan Fatty Acid Esters can contain impurities such as free acid and alcohol, arsenic (<3 ppm), lead (<10 ppm), and water (Elder 1985).

COSMETIC USE

The PEGs Sorbitan Beeswax are surfactant—emulsifying agents and surfactant—solubilizing agents in cosmetic formulations. Data submitted to the Food and Drug Administration (FDA) in 1998 indicated that PEG-20 Sorbitan Beeswax was used in 16 formulations in five product categories (Table 1), and the remaining PEGs Sorbitan Beeswax were not used (FDA 1998). Data provided by industry in 1998 and 1999 expanded the product categories in which PEG-20 Sorbitan Beeswax is reportedly used and provided current concentrations of use as shown in Table 2 (CTFA 1998a, 1998f, 1999a, 1999b). The highest currently reported concentration of use is 11% in blushers. These concentrations may be compared to data reported to FDA in 1984 indicating that PEG-6 and -20 Sorbitan Beeswax were used at concentrations $\leq$10% (FDA 1984).

TABLE 1
Frequency of use of PEG-20 Sorbitan Beeswax (FDA 1998)

Product category	Total no. of formulations in category	Total no. of formulations containing ingredient
Eyeliner	514	2
Mascara	167	8
Other Eye makeup preparations	120	1
Lipstick	790	4
Other Skin care preparations	692	1
1998 PEG-20 Sorbitan Beeswax total		**16**

GENERAL BIOLOGY

Absorption, Distribution, Metabolism, and Excretion

PEGs Sorbitan Beeswax

No information was available on the absorption, distribution, metabolism, or excretion of the PEGs Sorbitan Beeswax ingredients.

Polysorbates (PEG Sorbitan Fatty Acid Esters)

The ester link of the Polysorbate molecule was hydrolyzed by blood and pancreatic lipases following oral administration in labeling studies using rats. The fatty acid moiety was absorbed and metabolized as any other dietary fatty acid. The lauric acid moiety of PEG-20 Sorbitan Laurate was rapidly absorbed and oxidized by rats. After 24 hours, 75% to 80% of the lauric acid was expired as CO_2 and 4% was not absorbed from the alimentary tract. Twelve percent was found in the carcass, 2.5% in urine, and 1.2% in the liver. The polyoxyethylene sorbitan moiety was poorly absorbed from the gastrointestinal (GI) tract. Of the administered PEG group, 90% was excreted in the feces and 8% in the urine. In the case of the sorbitan moiety, 91% of the radioactivity was recovered in the feces, 2.1% in the urine, and 1.6% in the carcass. Similar results were observed following intravenous (IV) injection of PEG-20 Sorbitan Laurate (Elder 1984a).

PEGs

GI absorption of PEG is dependent on the molecular weight of the compound. In general, the greater the molecular weight of the PEG compound, the lesser the absorption that occurs. In both oral and IV studies, no metabolism was observed and the PEGs were rapidly eliminated unchanged in the urine and feces. In a study with human burn patients, monomeric ethylene glycol was isolated in the serum following topical exposure to a PEG-based antimicrobial cream, indicating that PEGs are readily absorbed through damaged skin (Andersen 1993).

Sorbitan Fatty Acid Esters

Sorbitan Stearate is hydrolyzed to stearic acid and anhydrides of sorbitol when ingested. Approximately 90% of the Sorbitan Stearate is absorbed and hydrolyzed when fed to rats in oil solution, and 50% is absorbed and hydrolyzed when fed as a water emulsion. Sorbitan Stearate does not accumulate to any appreciable amount (<0.5%) in the fat stores of the rat body (Elder 1985).

TABLE 2
Concentration of use of PEG-20 Sorbitan Beeswax

Product type	Reported concentration (%)	Reference(s)
Blushers	11	CTFA 1999a
Eyebrow pencil	3	CTFA 1999b
Eyelash primer	3	CTFA 1998a
Eyeliner	1.4–1.5	CTFA 1998a, 1999b
Eye makeup base	0.5	CTFA 1998a
Foot powders and sprays	1	CTFA 1999b
Lipstick	3–8	CTFA 1999a, 1999b
Makeup fixatives	0.2	CTFA 1999a, 1999b
Mascara	2–8	CTFA 1998a, 1999a, 1999b
Moisturizing creams, lotions, powders, and sprays	1	CTFA 1998f
Other makeup preparations	3	CTFA 1998f, 1999b

ANIMAL TOXICOLOGY

Acute Toxicity

PEGs Sorbitan Beeswax

PEG-6 Sorbitan Beeswax was administered via stomach intubation to two female rats (strain not stated) at a single dose of 10.0 g/kg. No signs of toxicity were noted, and none of the animals died prior to scheduled necropsy. Hydronephrosis and granular spleens were observed at necropsy. In a similar study, rats given 10.0 g/kg PEG-20 Sorbitan Beeswax had granular spleens and focal hemorrhages of the lungs. One rat had a scarlike lesion of the liver. Both compounds were classified as "practically nontoxic" (CTFA 1998b).

Sorbitan Fatty Acid Esters

Five female ddY mice were treated with a single oral dose of Sorbitan Sesquiisostearate at a volume of 10 ml/kg body weight. The acute oral LD_{50} was 25 ml/kg, which was considered "practically nontoxic" under the conditions of the study (CTFA 1998c).

PEGs

In general, PEGs have low oral and dermal toxicity. The greater molecular weight PEGs appear to be less toxic than the smaller molecular weight PEGs in oral studies. Inhalation of aerosolized PEG-75 (20% *w*:*w* in water) at concentrations up to 1008 mg/m^3 caused little or no toxicity in rats (Andersen 1993).

Polysorbates (PEGs Sorbitan Fatty Acid Esters)

Extensive acute and long-term oral toxicity testing in animals has produced evidence indicating the low order of toxicity after oral ingestion of the Polysorbates. Most of the reported toxicity can be attributed either directly or indirectly to the osmotic diarrhea caused by the polyoxyethylene sorbitan moiety retained within the intestinal lumen. Polysorbate 20 and product formulations containing 1.0% to 8.4% of Polysorbate 20, 40, 80, or 85 (PEG-20 Sorbitan Laurate, PEG-20 Sorbitan Palmitate, PEG-20 Sorbitan Oleate, or PEG-20 Sorbitan Trioleate, respectively) produced no evidence of acute or subchronic percutaneous toxicity, the only effects being erythema, edema, and desquamation at the site of application. Acute IV and intraperitoneal (IP) injections of the Polysorbates into rats or mice resulted in LD_{50} values indicative of a low order of parenteral toxicity. Injections of Polysorbates 60 (10 ml of 0.5% solution IV daily) and 80 (one 10-ml injection and one 15-ml injection daily IV of a 20% solution) into rabbits for up to 65 days produced lesions limited principally to the kidneys and monocyte-macrophage system (Elder 1984a).

Beeswax

Four of 10 rats died on day 2 of the 14-day observation period and the survivors had depression and ataxia after being dosed orally with undiluted Beeswax. In other studies, cosmetic formulations containing 0.3% to 13.0% Beeswax (100% or 33.3% in corn oil) were orally administered as 5 to 15 g/kg doses. No signs of toxicity were observed, and the LD_{50} values could not be computed (Elder 1984b).

Ten male Wistar rats fed 5 to 14.43 g/kg Synthetic Beeswax had chromorhinorrhea and chromodacryorrhea. Rats given 5 to 10.4 g/kg had diarrhea, ptosis, bulging eyes, and sniffling. One rat of the high dose group died on day 1, and another died on day 6 (Elder 1984c).

Sorbitan Fatty Acid Esters

The results of oral toxicity studies of Sorbitan Fatty Acid Esters indicated that these Sorbitans were relatively nontoxic via ingestion when administered at low concentrations. The lowest rat LD_{50} in the 20 sorbitan ester studies reported was 31 g/kg for Sorbitan Stearate (Elder 1985).

Subchronic Toxicity

Sorbitan Fatty Acid Esters

In subchronic feeding studies of Sorbitan Laurate in a variety of species (chickens, rats, monkeys, and hamsters), no toxic effects were noticed when the ester concentration in the feed was less than 10%. When the feed concentration was $\geq$10%, growth depression, decreased organ weights, diarrhea, unkempt appearance, hepatic and renal abnormalities, and GI tract irritation were generally observed. Subchronic feeding of Sorbitan Oleate to rats produced no abnormalities until the concentration of the ester was at least 10%. At this concentration, the same types of abnormalities occurred as those observed in the Sorbitan Laurate–fed animals (Elder 1985).

Chronic Toxicity

Sorbitan Fatty Acid Esters

Chronic feeding studies have been conducted with Sorbitans Stearate, Laurate, and Oleate. At a 5% dietary concentration, Sorbitan Laurate or Sorbitan Oleate produced no adverse effects when rats were fed the compounds for a 2-year period. Dogs fed 5% Sorbitan Stearate for 20 months had no compound-related changes. A feed concentration of $\geq$10% Sorbitan Stearate produced depressed growth and hepatic and renal abnormalities. Mice appeared more sensitive to toxic effects of Sorbitan Stearate than rats. A 0.5% dietary concentration produced growth depression in male rats, and a 4% dietary concentration produced renal abnormalities as well (Elder 1985).

Ocular Irritation

PEGs Sorbitan Beeswax

Undiluted PEG-6 Sorbitan Beeswax was nonirritating and 30% (in water) PEG-20 Sorbitan Beeswax was minimally irritating (score = 3.5/110) to the eyes of rabbits (CTFA 1998b).

An undiluted eyeliner containing 1.5% PEG-20 Sorbitan Beeswax was instilled three times into the conjunctival sac of three rabbits. Two days after instillation, one rabbit had redness, swelling, and/or discharge of the conjunctiva (Draize score = 2),

but no other reactions were observed. The eyeliner, therefore, was classified as minimally irritating to the eyes of rabbits. In studies using the same procedure, a mascara and lash conditioner containing 2% PEG-20 Sorbitan Beeswax were nonirritating and minimally irritating, respectively, to the eyes of three rabbits (CTFA 1998d).

A liquid eyeliner containing 1.5% PEG-20 Sorbitan Beeswax was tested for irritancy potential in the Eytex assay. The eyeliner was classified as a minimal irritant, and the equivalent Draize score was 1.2/110 (National Testing Corporation 1988).

PEGs

PEGs caused mild, transient ocular irritation in rabbits (Andersen 1993). The Polysorbates produced no more than minimal, transient ocular irritation in Draize rabbit eye irritation tests (Elder 1984a).

Beeswax

A cream formulation containing 6% Beeswax and 6% ceresin was evaluated for ocular irritancy using nine New Zealand white rabbits. The eyes of six rabbits were rinsed after instillation of the test material; at 24 hours, four had minimal chemosis and two had minimal conjunctival redness. No signs of irritation were observed in rabbits with unrinsed eyes (Elder 1984b).

A 0.1-ml volume of 3.0% Synthetic Beeswax was instilled into the conjunctival sac of three albino rabbits. No irritation was observed. In another study, six rabbits were treated with 0.1 ml of the compound. On days 1 to 3, the Draize scores were 6.3/110, 3/110, and 2/110, respectively. Synthetic Beeswax was deemed minimally irritating on days 1 and 2, and practically nonirritating on day 3 (Elder 1984c).

Sorbitan Fatty Acid Esters

Sorbitan Isostearate was nonirritating to the eyes of rabbits during two studies (Unichema International 1996). When 0.1 ml (10.0% in squalene) was tested using three male Japanese white rabbits, the average total score was 4.0/110.0, which corresponded to a grade of minimal irritant. Using the same procedure, Sorbitan Sesquiisostearate (10.0% in squalene) was a minimal irritant to the eyes of rabbits, with an average total score of 6.7/110.0 (CTFA 1998c).

Draize and Modified Draize ocular irritation studies using rabbits were performed. One study using a concentration of 30% Sorbitan Stearate was negative for ocular irritation, and low concentrations (4%) in products caused slight conjunctival irritation. High concentrations of Sorbitan Sesquioleate (3.0% to 100%) produced no ocular irritation. One study with Sorbitan Laurate (30%), and two studies each on Sorbitans Oleate (5% to 100%), Tristearate (30% to 40%), and Palmitate (4.0% to 30%) were negative for ocular irritation in the rabbit (Elder 1985).

Dermal Irritation and Sensitization

PEGs Sorbitan Beeswax

Undiluted PEG-6 Sorbitan Beeswax was nonirritating to the intact and abraded skin of New Zealand white rabbits when applied for 24 to 72 hours (CTFA 1998b). An eyeliner containing 1.5% PEG-20 Sorbitan Beeswax caused erythema and was minimally irritating in a single insult patch test using nine rabbits. The total primary irritation index (PII) score was 1.44/8.0. A mascara and lash conditioner containing 2.0% PEG-20 Sorbitan Beeswax each had PIIs of 1.33/8.0, and the formulations were classified as minimally irritating to the skin of six rabbits (CTFA 1998d).

Sorbitan Fatty Acid Esters

Sorbitan Isostearate was classified as a moderate irritant (primary irritation index, PII = 2.8/8.0) to the skin of rabbits. Sorbitan Isostearate also had very low sensitization potential when tested in four Magnusson-Kligman guinea pig maximization studies. The induction concentrations were 1% to 2% (intradermal injection) and 50% to 100% (topical application), and the challenge concentrations were 10% to 25%. In addition, a Landsteiner guinea pig test showed that intradermal injections of 0.2% Sorbitan Isostearate in propylene glycol caused mild to severe irritation in all animals, but did not cause sensitization reactions (Unichema International 1996).

Sorbitan Isostearate was described as "non-irritating, non-sensitizing, non-comedogenic in studies according to industry standard protocols (repeat-insult patch test [RIPT], comedogenicity)" and in the chorioallantoic membrane vascular assay, additional details were unavailable (CTFA 1998e).

The primary skin irritation potentials of Sorbitan Isostearate and Sorbitan Sesquiisostearate (both 10.0% in squalene) were evaluated using eight male Japanese white rabbits. The test materials were added to abraded and intact skin sites of the clipped back, and the sites were covered for 24 hours using patch-test plaster. The test sites were evaluated at 24 and 72 hours after administration of the test material. The PII's were 0.3/8.0 and 0.5/8.0, respectively, which corresponded to a grade of non- to weak irritant.

Sorbitan Isostearate and Sorbitan Sesquiisostearate were weak cumulative irritants using three male Hartley guinea pigs. A 0.05-ml volume of a 10% solution (in squalene) of each test substance was applied to the clipped and shaved skin of the flank, once daily for 3 consecutive days. The treatment sites were examined for signs of irritancy 24 hours after each application. The cumulative scores were 1.1/4.0 and 1.7/4.0, respectively (CTFA 1998c).

Numerous skin irritation studies in animals indicate that the Sorbitan Fatty Acid Esters are minimal to mild irritants. In acute skin irritation tests using rabbits, Sorbitan Stearate (1% to 60%) produced mild irritation. Sorbitan Laurate (1% to 100%) was mildly irritating to rabbit skin, causing dose-dependent erythema and edema. The rabbit dermal toxicity and irritation potential of Sorbitan Sesquioleate (3%) was minimal. Sorbitan Oleate (5% to 100%) was minimally irritating when applied to rabbit skin. When solutions of Sorbitan Oleate were applied to rabbit skin, erythema and edema developed. Sorbitan Palmitate (4% to 50%) when tested for acute dermal irritation in the rabbit

produced no irritation. A subchronic dermal study was negative for any systemic toxicity. Sorbitan Tristearate (30%) was nonirritating when applied to the skin of rabbits. Sorbitan Trioleate (1% to 100%) was generally found to be a skin irritant in rabbits. Sorbitan Trioleate when applied to rabbit skin produced erythema, edema, and thickening. No systemic toxicity was observed (Elder 1985).

PEGs

The PEGs were not irritating to the skin of rabbits or guinea pigs. PEG-75 was not a sensitizer (Andersen 1993).

Polysorbates (PEG Sorbitan Fatty Acid Esters)

The Polysorbates had little potential for rabbit and mouse skin irritation in acute studies. The Polysorbates that were tested in subchronic skin irritation tests for up to 60 days produced local skin reactions ranging from minimal inflammation to necrosis. These changes were attributable to damage of epidermal cell membranes by the emulsifying action of the Polysorbates. Moderate to strong skin sensitization to Polysorbate 20 was observed in one Magnusson-Kligman guinea pig maximization test. In another guinea pig skin sensitization assay, no skin sensitization to Polysorbates 65 (PEG-20 Sorbitan Tristearate) and 80 was observed (Elder 1984a).

Beeswax

When 5 g Synthetic Beeswax (in 1 ml corn oil) was applied to intact and abraded skin sites of six New Zealand white rabbits for 24 hours, the Draize score (at 72 hours) was 2.08/8.0. In a second primary irritation assay, 0.5 ml of the test compound was applied under occlusive patches to abraded and intact skin sites of three albino rabbits. The Draize primary irritation index was 0.0/8.0.

Fifty percent Synthetic Beeswax (in distilled water) with 1% carboxymethyl cellulose and 0.2% Polysorbate 80 was applied to the clipped backs of guinea pigs (number not available) for 3 consecutive days/week for 3 weeks. One application was made in the fourth week. The volume of the first application was 0.5 ml, and the volume of the remaining nine applications was 0.1 ml. Challenge occurred at 14 days after the last application. The scores were 0.16/4.0 (erythema) and 0.05/4.0 (edema). The investigators concluded that the test material had no potential for irritation or sensitization (Elder 1984c).

Other Safety Tests

PEGs Sorbitan Beeswax

A chorioallantoic membrane vascular assay was performed on 50% (aqueous) and 100% concentrations of a cosmetic formulation containing 1.5% PEG-20 Sorbitan Beeswax. Ten eggs per group were treated with the test materials, incubated for 30 minutes, and were examined for signs of vascular hemorrhage, capillary injection, or ghost vessels. The RC_{50} was >100% (MB Research Labs 1991).

Polysorbates (PEG Sorbitan Fatty Acid Esters)

Polysorbate 80 (PEG-20 Sorbitan Oleate) produced superficial, mild damage to the intestinal mucosae of rabbits and rats. Polysorbate 20 (PEG-20 Sorbitan Laurate) produced no inflammation when infused into the guinea pig urinary bladder (Elder 1984a).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

PEGs Sorbitan Beeswax

Data on the reproductive or developmental toxicity of the PEGs Sorbitan Beeswax ingredients were not found. Data on PEGs and Sorbitol are provided. Because of concerns about the reproductive and developmental toxicity of the PEG monomer, ethylene glycol, a separate section is included to address that issue.

PEGs

No adverse reproductive effects occurred during subchronic (90 days) and chronic (2 years) oral toxicity studies of PEG-6-32 and PEG-75. In the subchronic study, PEG-75 was tested at a dose of 0.23 g/kg/day. In the chronic study, PEG-75 was tested at doses up to 0.062 g/kg/day and, PEG-6-32, at doses up to 1.69 g/kg/day (Andersen 1993).

Sorbitol

MacKenzie et al. (1986) performed a multigeneration feeding study to determine the reproductive and developmental effects of Sorbitol. Twelve male and 24 female Charles River CD (SD) BR rats per group were fed diets having 2.5%, 5.0%, or 10% Sorbitol (replacing the sucrose content of the basal feed) during a 96-week multigeneration study. The two high concentrations were "built up in 2.5% steps at weekly intervals." The F_0 rats were mated to produce the F_{1a} and F_{1b} litters. The F_{1b} rats were treated and mated to produce the F_{2a} and F_{2b} litters. The F_{2b} rats were treated and mated to produce the F_{3a} litters. Twelve rats/sex/group were fed the test diets for 4 weeks, then were killed. Gross examinations were performed on all mated animals and two rats/sex of the F_{1a} and F_{2a}. Gross and microscopic examinations and biochemical analyses were performed on the F_{3a} rats. In this study, the feeding of up to 10% Sorbitol to rats had no significant adverse clinical, behavioral, or reproductive effects, and no significant gross or microscopic changes were observed.

The safety of hydrogenated starch hydrolysates (HSH), which are mixtures of polyhydric alcohols such as ~7.0% Sorbitol, was investigated using a 2-year ingestion study (50 Sprague-Dawley rats/sex/group), a multigeneration reproduction study (20 rats/sex/group), and a teratology study (30 dams/group). At a concentration of 18% in drinking water (3000 to 7000 mg/kg/day), HSH did not produce reproductive or developmental effects (Modderman 1993).

Ethylene Glycol and Its Ethers

It is generally recognized that the PEG monomer, ethylene glycol, and certain of its monoalkyl ethers (e.g., methoxyethanol,

a.k.a. ethylene glycol monomethyl ether) are reproductive and developmental toxins. The CIR Expert Panel undertook a separate, limited scope review of these compounds in order to assess the possibility that PEG-derived cosmetic ingredients could present similar concerns (Andersen 1999). In summary, this report concluded that the ethylene glycol monoalkyl ethers are not themselves toxic, but rather that one or more alcohol or aldehyde dehydrogenase metabolites are toxic. From the available data, the report also concluded that the toxicity of the monoalkyl ethers is inversely proportional to the length of the alkyl chain (methyl is more toxic than ethyl than propyl than butyl, etc.).

The PEGs Sorbitan Beeswax are chemically different from the alkyl ethers; therefore, the Panel concluded that no reproductive or developmental hazard is posed by those compounds.

GENOTOXICITY

PEGs Sorbitan Beeswax

No data were available on the mutagenicity of the PEGs Sorbitan Beeswax ingredients.

PEGs

PEG-8 was negative in a Chinese hamster ovary cell mutation test, sister-chromatid exchange test, and unscheduled DNA synthesis assay. PEG-150 was not mutagenic in a mouse $TK^{+}/^{-} \rightarrow TK^{-}/^{-}$ forward mutation assay. The mutation index ranged from 0.8 to 2.3 (Andersen 1993).

Polysorbates (PEG Sorbitan Fatty Acid Esters)

Sorbitan Stearate was not mutagenic in bacteria with or without metabolic activation. Sorbitan Stearate did not transform primary Syrian golden hamster embryo cells in vitro. Sorbitan Oleate at a concentration of 0.01% inhibited in vitro DNA repair (Elder 1985).

Polysorbate 80 (PEG-20 Sorbitan Oleate) was nonmutagenic in the Ames and micronucleus tests (Elder 1984a).

An unspecified Sorbitan Fatty Acid Ester (maximum dose = 5.0 mg/plate, in dimethyl sulfoxide (DMSO)) was negative for mutagenicity in the Ames test using *Salmonella typhimurium* strains TA92, TA94, TA98, TA100, TA1535, and TA1537. In the chromosomal aberrations test using Chinese hamster fibroblasts, a maximum dose of 0.3 mg/ml of the test compound (in DMSO) resulted in 5.0% polyploid cells and 8.0% structural aberrations 48 hours after treatment. The results were considered equivocal, and polyploidization effects were observed (Ishidate et al. 1984).

PEG-20 Sorbitan Stearate was not mutagenic in *S. typhimurium* strains TA100 and TA98; the Polysorbate also did not induce in vitro transformation of hamster embryo cells. But in a study examining the role of inhibition of DNA repair as a mechanism in cocarcinogenesis, Sorbitan (0.01%) was found to inhibit the repair of UV-irradiated DNA extracted from normal human lymphocytes (Elder 1985).

Sorbitol

After being fed to adult *Drosophila*, Sorbitol was negative for whole-chromosome loss and did not cause clastogenic effects or nondisjunction. In these studies, Sorbitol did not appear to cause sex-linked recessive lethals; however, it could not be classified as either positive or negative for mutagenic activity due to an inadequate sample size (Abbott and Bowman 1976).

Chinese hamster ovary cells in medium made hyperosmotic with Sorbitol had significant increases in the incidence of chromosomal aberrations. The test concentrations were 300 to 450 mM. The cells were harvested for aberration analysis 24 to 26 hours after the beginning of the 4-hour treatment period. Cells treated with 300 to 350 mM Sorbitan had 100% survival, and cells treated with 400 and 450 mM had 40% and 15% survival, respectively. Survival was measured after 6 days of colony formation, as a percentage of the untreated control value. The numbers of aberrations per 100 cells were 2 (control), 26 (300 mM; one cell was excluded), 11 (350 mM), 29 (400 mM), and 27 (450 mM; only 30 scoreable cells). The incidences of cells with aberrations were 2% (control), 8% (300 mM), 7% (350 mM), and 17% (400 and 450 mM). The investigators concluded that the increase in aberrations represented an indirect effect on the cells (Galloway et al. 1987).

The addition of sugars such as Sorbitol reduced the mutagenicity of smoke condensates of high- and low-tar cigarettes, as tested using *S. typhimurium* strains TA98 and TA100, with metabolic activation. Cigarettes treated with Sorbitol yielded more tar than untreated cigarettes. When 0.51 g Sorbitol was added to each high-tar cigarette, the percent mutagenicity per mg smoke condensate was 66% (TA100) and 37% (TA98) relative to cigarettes without added sugars. The percent mutagenicity per cigarette was 77% (TA100) and 46% (TA98). When 0.70 g Sorbitol was added to low-tar cigarettes, the percentages were 65% (TA100) and 23% (TA98) per milligram smoke condensate and 184% (TA100) and 66% (TA98) per cigarette. The addition of sugars without metabolic activation had no effect on mutagenicity of the cigarette smoke condensates (Sato et al. 1979).

CARCINOGENICITY

PEGs Sorbitan Beeswax

No data were available on the carcinogenicity of the PEGs Sorbitan Beeswax ingredients.

PEGs

PEG-8 was not carcinogenic when administered orally, intraperitoneally, or subcutaneously to various test animals (Andersen 1993).

Polysorbates (PEG Sorbitan Fatty Acid Esters)

The Polysorbates have been found in numerous studies to be noncarcinogenic when administered to laboratory animals, although Polysorbate 80 produced some neoplastic changes in

mixed mouse epidermal and dermal in vitro tissue cultures (Elder 1984a). In multiple studies, the Polysorbates have enhanced the activity of known chemical carcinogens while not actually being carcinogenic themselves. Proposed mechanisms of this tumor enhancement included induction of cellular hyperproliferation or inhibition of DNA repair. The Polysorbates also have tumor growth inhibition activity under certain conditions (Elder 1984a).

Mice fed low concentrations of Sorbitan Stearate for 80 weeks had no difference in tumor type and incidence as compared to control animals (Elder 1985). Sorbitan Laurate was inactive as a carcinogen when painted on mouse skin for 73 weeks (Elder 1985).

Sorbitol

At a concentration of 18% in drinking water (3000 to 7000 mg/kg/day), hydrogenated starch hydrolysates (mixtures of polyhydric alcohols such as ~7.0% Sorbitol) did not produce evidence of carcinogenicity after 2 years of treatment. This study used 50 Sprague-Dawley rats/sex/group. No significant clinical signs of toxicity were observed (Modderman 1993).

In studies using rats, high dietary concentrations of Sorbitol caused enlargement of the cecum, increased absorption of calcium from the gut, increased urinary excretion of calcium, pelvic and corticomedullary nephrocalcinosis, acute tubular nephropathy, urinary calculus formation, and hyperplasia and neoplasia of the adrenal medulla. The investigator concluded that adrenal neoplasms observed in mice fed 20% Sorbitol were laboratory artifacts, and not indicative of human risk exposed to normal concentrations of Sorbitol in the diet (Roe 1984).

Cocarcinogenicity

PEGs Sorbitan Beeswax

No data were available on the cocarcinogenicity of the PEGs Sorbitan Beeswax ingredients.

Polysorbates (PEG Sorbitan Fatty Acid Esters)

Sorbitan Laurate applied twice weekly for 75 weeks to the skin of mice after an initiating exposure to 7,12-dimethylbenz(a) anthracene (DMBA) produced more tumors than did DMBA treatment alone. A comparison of Sorbitan Laurate, Sorbitan Oleate, and Sorbitan Trioleate was done in a similar mouse study. DMBA at 0.3%, 0.03%, and 0.003% was used as the tumor initiator, followed by skin treatment with one of the sorbitan fatty acid esters, once or twice daily, 6 days a week, for 52 weeks. With 0.3% DMBA, Sorbitan Laurate produced more tumors than did DMBA treatment alone, but neither Sorbitan Oleate nor Sorbitan Trioleate promoted DMBA carcinogenesis. With 0.03% DMBA, none of the sorbitan fatty acid esters were tumor promoters. With 0.003% DMBA, Sorbitan Laurate and Sorbitan Trioleate produced more tumors than did DMBA treatment alone, but Sorbitan Oleate was not a tumor promoter (Elder 1985).

CLINICAL ASSESSMENT OF SAFETY

Oral Toxicity

The Polysorbates have been ingested by human beings in situations ranging from an accidental administration of 19.2 g of Polysorbate 80 (PEG-20 Sorbitan Oleate) to an infant on 2 consecutive days to daily therapeutic administration of up to 6.0 g of Polysorbate 80 to adults for up to 4 years. In these studies, oral ingestion of the Polysorbates produced little or no adverse effects (Elder 1984a).

Dermal Irritation and Sensitization

PEG-6 and -20 Sorbitan Beeswax (concentrations not specified) were nonsensitizing when patch-tested in 50 subjects. The test materials were applied to 1×1-inch cotton twill pads that were affixed to the skin for 72 hours using 2×2-inch elastic adhesive patch. Seven days after patch removal, the test compounds were reapplied in the same fashion; no reactions were observed (CTFA 1998b).

An undiluted liquid eyeliner containing 1.5% PEG-20 Sorbitan Beeswax was tested for primary irritancy using 17 subjects. The PII was 0.00, and the formulation was considered nonirritating in the single insult patch test.

In a cumulative irritation study using 12 subjects, a volume of 0.3 ml of the same eyeliner was applied to the skin of the back under a closed patch with Webril pad. Applications were made daily for 21 consecutive days. The total scores were 299/756 (base $n = 10$) and 357/630 (base $n = 12$), and the irritating potential for the formulation was classified as "possibly mild in normal use" (CTFA 1998d).

A mascara containing 2.0% PEG-20 Sorbitan Beeswax was tested for irritancy in a 4-day minicumulative patch test. The PII was 0.24/8.0, and the formulation was classified as mild. The number of subjects was not available. In similar studies, a lash primer/conditioner containing 3.0% PEG-20 Sorbitan Beeswax was "adequately mild when used in a conventional manner," and had a PII of 0.25/8.0. to 0.34/8.0 (CTFA 1998d).

The eyeliner containing 1.5% PEG-20 Sorbitan Beeswax was also tested for sensitization using 94 subjects, 4 of whom withdrew from the study for reasons unrelated to the test material. The test sample was applied to the skin of the upper back under a Webril pad affixed to an adhesive bandage and secured with Scanpor tape. Induction applications were made for 24 hours, three times weekly for 3 consecutive weeks. Challenge applications were made to untreated skin sites in weeks 6 to 7. The patches were removed 24 hours later and reactions were scored 24 and 48 hours after patch removal. Two subjects had possible sensitization reactions and were repatched for 24 hours with the test formulation: one subject received the eyeliner as is, and one was repatched with a 50% aqueous dilution of the formulation. The investigators concluded that the eyeliner was nonsensitizing under the conditions of this study (CTFA 1998d).

In a similar sensitization study, a mascara containing 2.0% PEG-20 Sorbitan Beeswax was tested using 89 subjects, 2 of

whom withdrew for reasons unrelated to the test procedure. Under the conditions of the study, the mascara was not a sensitizer (CTFA 1998d).

A lash conditioner containing 2.0% PEG-20 Sorbitan Beeswax was nonirritating and nonsensitizing when tested in an RIPT using 86 subjects (Hill Top Research, Inc. 1988).

A liquid eyeliner containing 1.5% PEG-20 Sorbitan Beeswax was tested for irritancy of the eye area during normal use by 56 subjects. One half of the panelists used the test formulation and one half used a control formulation. After 3 weeks of use, the two groups switched formulations for another three weeks. Dermatologic examinations of the ocular area (browline, suborbital area, lids and lid margins, and outer aspects of the eye) were performed at weeks 3 and 6. One panelist had slight scaling on the lid margins after initially using the test eyeliner, but had no subjective discomfort. The eyeliner "[did] not have the potential to evoke adverse effects on the eye area when used under consumer use conditions" and was "very to moderately gentle to the eyes." Similar results were reported for a cream mascara containing 2.0% PEG-20 Sorbitan Beeswax (CTFA 1998d).

The potential of a lash primer/conditioner containing 2.0% PEG-20 Sorbitan Beeswax to cause irritation and subjective discomfort was determined using 19 panelists who used the product prior to application of mascara for 5 consecutive days. No clinical irritation was observed for the eye area exposed to the conditioner, with or without the mascara, and minimal discomfort was reported (CTFA 1998d).

PEGs

Cases of systemic toxicity and contact dermatitis in burn patients were attributed to a PEG-based topical ointment. In clinical studies, PEG-6 and PEG-8 caused mild immediate hypersensitivity. However, PEG-6, -8, -32, and -75 were not sensitizers (Andersen 1993).

Polysorbates (PEG Sorbitan Fatty Acid Esters)

In extensive clinical skin testing using the Schwartz prophetic patch test, the Polysorbates had little potential for human skin irritation and produced no evidence of skin sensitization in 580 subjects. When 1206 patients with eczema were tested in a chamber method 24-hour occlusive patch test for allergic contact dermatitis to a mixture of 5% Polysorbate 60 (PEG-20 Sorbitan Stearate) and 5% Polysorbate 80 (PEG-20 Sorbitan Oleate) in petrolatum, reactions were observed in only 2 of the patients (<0.2%). Several product formulations containing Polysorbates have been tested for human skin sensitization using 3481 subjects and a variety of testing methods; no reactions indicative of sensitization were found to any of the Polysorbates in these assays. Investigations using patients known to have skin disease have produced isolated instances of skin sensitization to Polysorbate 40 (PEG-20 Sorbitan Palmitate) or Polysorbate 80. Polysorbate 80 (IV) produced hemodynamic changes in five patients (Elder 1984a).

Results from three RIPTs (involving a total of 420 subjects) indicated that Sorbitan Stearate was not a sensitizer. Products containing low concentrations of Sorbitan Stearate were mild irritants in 21-day cumulative irritation studies (Elder 1985).

In a Schwartz prophetic patch test, Sorbitan Laurate produced no irritation. Results of human skin tests for sensitivity to Sorbitan Sesquioleate indicated that the compound was a nonsensitizer. In two Schwartz prophetic patch tests (60 subjects total) utilizing 30% and 100% concentrations, Sorbitan Sesquioleate produced no reactions. The results of five RIPTs involving 352 subjects indicated that none of the five products containing 1% to 3% Sorbitan Sesquioleate produced sensitization; however, some subjects experienced mild irritation (Elder 1985).

Several products containing 1.75% to 2.0% Sorbitan Oleate have been tested using human subjects. In four 21-day cumulative irritation studies, the products tested were mildly irritating. In the tests using entire product formulations, the specific ingredient(s) causing irritation was not determined. Four RIPTs involving 339 subjects classified the Sorbitan Oleate–containing products as nonsensitizers. No irritation was observed in maximization tests. A product usage test on 53 subjects produced mild irritation in two individuals (Elder 1985).

In a Schwartz prophetic patch test using Sorbitan Tristearate, 211 panelists had no signs of irritation. Sorbitan Palmitate–containing skin products were slightly irritating to the skin of humans in 21-day cumulative irritation tests (34 subjects total). In a Shelanski/Jordan RIPT (206 subjects), a skin care product containing Sorbitan Palmitate was nonirritating and nonsensitizing. Several products containing 5% Sorbitan Trioleate were tested on human subjects. Sorbitan Trioleate–containing products were slightly irritating in 21-day cumulative irritation tests, Shelanski/Jordan RIPT, Modified Schwartz-Peck predictive patch tests, and in a 4-week usage test (Elder 1985).

Sorbitan Isostearate (2.5%) was tested in a RIPT using 201 subjects. During the induction period 48- to 72-hour occlusive patches containing 0.2 g of the test material were applied to the upper arm or back. Patches were applied three times per week for 3 weeks. After a 2-week nontreatment period, a 72-hour challenge patch was applied to a previously unexposed site. Reactions were scored at 96 hours post application. Sorbitan Isostearate did not induce a sensitization response (CTFA 1998e).

A 24-hour occlusive patch test was performed using 56 subjects. A 0.05-ml volume of Sorbitan Isostearate (10.0% in squalene) was applied to the intact skin of the forearm for 24 hours and then the treatment site was examined for signs of primary irritation. None of the subjects reacted to Sorbitan Isostearate under the conditions of this study. Sorbitan Sesquiisostearate (10.0% in squalene) was evaluated similarly using 10 subjects, none of whom reacted to the test material (CTFA 1998c).

A 24-hour occlusive patch test using 56 subjects exposed to 10% Sorbitan Isostearate produced no signs of irritation. In a similar study in 10 subjects, 10% Sorbitan Sesquiisostearate

produced no irritation. Sorbitan Isostearate at a concentration of 2.5% was negative in an RIPT using 201 subjects (CIR 1999).

Beeswax

The total irritation score of a cream formulation containing 6% Beeswax and 6% Synthetic Beeswax was 6.4/630 for a 21-day cumulative irritation study using 14 subjects. No irritation was observed when 100 women used the formulation daily for 14 days. A Schwartz-Peck prophetic patch test of the above formulation was performed using 98 subjects. Two applications (at 48-hour intervals) were made using open and closed patches. After the second application, photosensitization potential was evaluated using irradiation from a solar simulator at a distance of 12 inches for 1 minute. No irritation, sensitization, or photosensitization reactions were observed. No irritation, sensitization, or photosensitization was observed in 49 subjects exposed to the above formulation during a Draize-Shelanski RIPT, followed by UV irradiation (360 nm). No evidence of contact sensitization was observed when 22 subjects were treated (volar forearm skin sites) with 5% sodium lauryl sulfate 24 hours prior to treatment with the above formulation under occlusive conditions at 48-hour intervals. Challenge took place 10 to 14 days after the last application (Elder 1984b).

No irritation or sensitization was observed after a lipstick (7.2% to 9.4% Synthetic Beeswax) was tested in 896 subjects using an RIPT.

Comedogenicity

A product containing 5% Sorbitan Isostearate was tested using 20 human subjects to determine its comedogenicity. Reactions that scored a value of 1 or greater, and were statistically different from the negative control, were considered positive for comedogenicity. Data from the global assessment of the test and the control values were compared statistically to determine biological significance ($p \leq .05$). No significant clinical irritation was observed during the study period. Reactions ranging from +0.5 to +1.0 were observed occasionally in 9 of the 20 subjects. Comparison of the test sites and untreated control sites through statistical analysis for the formation of microcomedones yielded a p value of greater than .05. It was concluded that this product did not produce evidence of comedogenicity (CTFA 1998e).

Photosensitization

In studies involving exposure to UV light, no evidence of photocontact sensitization to the Polysorbates was observed, although isolated instances of mild irritation occurred following UV exposure after application of formulations containing the Polysorbates (Elder 1984a).

A formulation containing 6% Beeswax and 6% ceresin produced no evidence of photosensitization potential during a Schwartz-Peck prophetic patch test or an RIPT (see "Dermal Irritation and Sensitization") (Elder 1984b).

No photosensitization reactions were observed when 7.2% to 9.4% Synthetic Beeswax in a lipstick was applied to the skin of 83 subjects. The treated skin sites were irradiated with a 150-W solar simulator set at continuous emission of 290 to 400 nm. The treatment and UV exposure were repeated six times, and challenge application was made at 10 days (Elder 1984c).

Photosensitization assessments of products containing Sorbitan Stearate or Sorbitan Oleate classified both products as nonphototoxic and nonphotoallergenic. Sorbitans Laurate, Sesquioleate, Palmitate, and Trioleate did not absorb radiation in the UVA and UVB range in ultraviolet spectral analysis (Elder 1985).

Ocular Irritation

Seventy-five subjects used a lash primer/conditioner containing 2.0% PEG-20 Sorbitan Beeswax for 6 weeks prior to mascara application. Ophthalmologic examinations were performed initially and after completion of the study. No irritation was observed and the potential to cause subjective discomfort was low (CTFA 1998d).

SUMMARY

PEG-6, -8, and -20 Sorbitan Beeswax are ethoxylated derivatives of Beeswax that function as surfactants in cosmetic formulations. In 1998, PEG-20 Sorbitan Beeswax was reported used in 16 cosmetic formulations; PEG-6 and -8 Sorbitan Beeswax were not reported used. Data submitted by industry indicated that PEG-20 Sorbitan Beeswax was used at concentrations from 0.2% in make-up fixatives to 11% in blushers. In 1984, it was reported used at concentrations $\leq$10%.

Few data were available on the PEGs Sorbitan Beeswax. Toxicology data on Beeswax, Synthetic Beeswax, Sorbitan Esters, PEGs, and Polysorbates were reviewed as a further basis for the assessment of safety.

The ester link of the Polysorbate (PEG Sorbitan Fatty Acid Ester) molecule was hydrolyzed by blood and pancreatic lipases after oral administration. The fatty acid moeity was absorbed and metabolized as any other dietary fatty acid, and the PEG Sorbitan moiety was poorly absorbed from the GI tract. GI absorption of PEG was inversely related to the molecular weight of the compound. PEGs are readily absorbed through damaged skin. Sorbitan Stearate was hydrolyzed to the stearic acid and anhydrides of sorbitol, and did not accumulate in the fat stores of the rat.

PEG-6 Sorbitan Beeswax was "practically nontoxic" when rats were treated with doses of 10.0 g/kg during acute IP studies. PEGs had low oral, dermal, and inhalation toxicity; greater molecular weight PEGs were less toxic than smaller molecular weight PEGs. The Polysorbates were not toxic during acute and long-term feeding studies, or during acute and short-term IV and IP injection studies. Formulations containing the Polysorbates produced no evidence of acute or subchronic percutaneous toxicity. Formulations containing up to 13% Beeswax (5 to 15 g/kg

doses) were not toxic to rats. Undiluted Beeswax killed 2 of 10 rats within 2 days during an acute oral toxicity study. Ten rats fed 5 to 14.4 g/kg Synthetic Beeswax had chromorhinorrhe a and chromodacryorrhea; rats fed 5 to 10.4 g/kg had diarrhea, ptosis, bulging eyes, and sniffling. Two rats died after ingestion of the high dose.

The Sorbitan Esters (<10%) were relatively nontoxic via ingestion. The lowest LD_{50} (rats) reported was 31 g/kg Sorbitan Stearate. No adverse effects were observed when rats, mice, and dogs were fed 5% Sorbitans Laurate, Oleate, and Stearate for up to 2 years. In other studies, the feeding of 0.5%, 4%, and 10% Sorbitan Stearate to mice and rats resulted in depressed growth and renal and/or hepatic abnormalities.

Undiluted PEG-6 Sorbitan Beeswax was nonirritating to the eyes of rabbits, and a 30% aqueous solution of PEG-20 Sorbitan Beeswax was minimally irritating (Draize score = 3.5/110). Eye makeup formulations containing 1.5% to 2.0% PEG-20 Sorbitan Beeswax were non- to minimally irritating to the eyes of rabbits. PEGs, Polysorbates, Sorbitan Esters, Beeswax, and Synthetic Beeswax were non- to mild ocular irritants.

Undiluted PEG-6 Sorbitan Beeswax was nonirritating to the intact and abraded skin of rabbits. Cosmetic formulations containing 1.5% to 2.0% PEG-20 Sorbitan Beeswax were non- to minimal irritants to the skin of rabbits. The PEGs were not irritating to the skin of rabbits or guinea pigs, and PEG-75 was not a sensitizer. The Polysorbates had little potential for rabbit and mouse skin irritation during acute studies. Polysorbate 20 was a moderate to strong sensitizer in one study using guinea pigs, and Polysorbates 65 and 80 were nonsensitizers. Synthetic Beeswax (5 g in 1 ml corn oil) had Draize scores of 0 to 2.08 (out of 8.00) during primary irritation studies using rabbits. At a concentration of 50% in water, Synthetic Beeswax was nonsensitizing to guinea pigs. Sorbitan Esters (3% to 100%) were minimal to mild irritants.

Ethylene glycol and certain of its monoalkyl ethers are reproductive and developmental toxins. As PEGs Sorbitan Beeswax are chemically different from these ethers, reproductive and developmental toxicity due to the ethers was not of concern. PEGs did not cause adverse reproductive effects during subchronic and chronic feeding studies.

PEG-8 and -150 were not mutagenic in several genotoxicity assays. Polysorbate 80 was nonmutagenic in the Ames test. sorbitan Stearate was not mutagenic in tests using bacteria, with or without metabolic activation, and did not transform hamster embryo cells in vitro. Sorbitan Oleate (0.01%) inhibited in vitro DNA repair. PEG-8 was not carcinogenic during oral, IP, or subcutaneous (SC) administration. The Polysorbates were generally noncarcinogenic, but enhanced the activity of some known chemical carcinogens. Sorbitan Stearate was not carcinogenic in mice during a feeding study, but Sorbitan Laurate was a tumor promoter during a mouse skin-painting study. Sorbitans Oleate and Trioleate were inactive as tumor promoters. In another study, undiluted Sorbitans Laurate and Trioleate were not cocarcinogens.

In clinical studies, PEG-6 and -20 Sorbitan Beeswax were nonsensitizers. Formulations containing up to 3.0% PEG-20 Sorbitan Beeswax were mildly irritating and nonsensitizing during in-use, minicumulative, and RIPTs. Systemic toxicity and contact dermatitis were observed in burn patients treated with PEG-containing ointments, but PEGs were not sensitizing to normal skin. The Polysorbates and Sorbitan Esters were nontoxic after oral ingestion. Polysorbates, Beeswax, and Synthetic Beeswax did not cause irritation, sensitization, or photosensitization. The Sorbitan Esters were minimal to mild skin irritants in humans, but were nonsensitizing, nonphototoxic, and nonphotoallergenic.

DISCUSSION

Because there were few data available on the PEGs Sorbitan Beeswax ingredients, the available data from previous safety assessments of Beeswax, Synthetic Beeswax, Sorbitan Esters, PEGs, and PEG Sorbitan fatty acid esters, also known as Polysorbates, was discussed primarily.

Data summarized in this report indicate that Beeswax did not produce any mutagenicity or toxicity in rats and in skin and eye irritation tests it produced minimal to no irritation in rabbits. Beeswax also did not cause any phototoxic reactions in hairless mice, swine, and humans. Subchronic dermal toxicity tests in rabbits and rats produced no topical or systemic effects. In clinical studies, a 21-day cumulative patch test and an RIPT containing 6% Beeswax and 6% Synthetic Beeswax caused no irritation. Based on this data, the Expert Panel determined Beeswax to be safe as used in cosmetics under present practices of concentration and use. The Expert Panel believes the information from the Beeswax report supports a safe as used conclusion for PEG -6, -8, and -20 Sorbitan Beeswax.

The CIR Expert Panel, however, was concerned about the sensitization and toxicity potential of the PEGs Sorbitan/Sorbitol Fatty Acid Esters when applied to damaged skin. This concern arose because of positive patch tests and incidences of nephrotoxicity in burn patients treated with an antimicrobial cream that contained PEG-6, PEG-20, and PEG-75. PEG was the causative agent in both animal and human studies; no evidence of systemic toxicity or sensitization was found in studies with intact skin. The cosmetics industry should consider this information when formulating products with PEGs Sorbitan/Sorbitol Fatty Acid Esters.

Also of concern to the Expert Panel was the possible presence of 1,4-dioxane and ethylene oxide impurities. The Panel stressed that the cosmetics industry should continue to use the necessary procedures to remove these impurities from the PEGs Sorbitan/Sorbitol Fatty Acid Ester ingredients before blending them into cosmetic formulations.

The Expert Panel considered the finding that treatment of normal, human lymphocytes with 0.01% Sorbitan Oleate reduces DNA repair following UV irradiation, and the researchers' hypothesis that this effect could be a mechanism in

cocarcinogenesis. The Panel carefully considered the data on the cocarcinogenesis of the Sorbitan Esters, noting the high exposure levels used, the high frequency of exposure, and the lack of a dose-response, and concluded that the positive response in these studies does not constitute a risk in cosmetic formulations.

CONCLUSION

Based on the available data on the ingredients themselves and on data on the components, the Expert Panel concludes that PEG -6, -8, and -20 Sorbitan Beeswax are safe for use as cosmetic ingredients under the present practices of use. The Expert Panel recommends that cosmetic formulations containing PEG-6, PEG-20, or PEG-75 not be used on damaged skin.

REFERENCES

Abbott, D. M., and J. T. Bowman. 1976. A test of the mutagenic effects of sorbitol in *Drosophila melanogaster*. *J. Food Sci*. 41:572–574.

Andersen, F. A., ed. 1993. Final report on the safety assessment of Polyethylene Glycols (PEGs) -6, -8, -32, -75, -150, -14M, -20M. *J. Am. Coll. Toxicol*. 12:429–457.

Andersen, F. A., ed. 1999. Special report on the reproductive and development toxicity of Ethylene Glycol and its ethers. *J. Am. Coll. Toxicol*. 18:53–67.

Cosmetic Ingredient Review (CIR). 1999. Final report of the safety assessment of Sorbitan Caprylate, Sorbitan Cocoate, Sorbitan Diisostearate, Sorbitan Dioleate, Sorbitan Distearate, Sorbitan Isostearate, Sorbitan Olivate, Sorbitan Sesquiisostearate, Sorbitan Sesquistearate, and Sorbitan Triisostearate. Washington: CIR (28 pages.)[2]

Cosmetic, Toiletry, and Fragrance Association (CTFA). 1998a. Information on use concentrations. Unpublished data submitted by CTFA, 1-21-98. (2 pages.)[2]

CTFA. 1998b. Data on PEGs Sorbitan Beeswax. Unpublished data submitted by CTFA, 1-21-98. (5 pages.)[2]

CTFA. 1998c. Data on Sorbitan Isostearate and Sorbitan Sesquiisostearate. Unpublished data submitted by CTFA, 7-16-98. (11 pages.)[2]

CTFA. 1998d. Data on PEGs Sorbitan Beeswax. Unpublished data submitted by CTFA, 6-26-98. (94 pages.)[2]

CTFA. 1998e. Unpublished data on Sorbitan Isostearate, submitted 6-22-98. (2 pages.)[2]

CTFA. 1998f. Unpublished data on PEG-20 Sorbitan Beeswax, submitted 11/18/98. (1 page.)[2]

CTFA. 1999a. Unpublished data on PEG-20 Sorbitan Beeswax, submitted 4/29/99. (2 pages.)[2]

CTFA. 1999b. Unpublished data on PEG-20 Sorbitan Beeswax, submitted 16 June 1999. (2 pages.)[2]

Elder, R. L., ed. 1984a. Final report on the safety assessment of Polysorbates 20, 21, 40, 60, 61, 65, 80, 81, and 85. *J. Am. Coll. Toxicol*. 3:1–82.

Elder, R. L., ed. 1984b. Final report on the safety assessment of Candelilla Wax, Carnauba Wax, Japan Wax, and Beeswax. *J. Am. Coll. Toxicol*. 3: 1–41.

Elder, R. L., ed. 1984c. Final report on the safety assessment of Fossil and Synthetic Waxes. *J. Am. Coll. Toxicol*. 3:43–99.

Elder, R. L., ed. 1985. Final report on the safety assessment of Sorbitan Stearate, Sorbitan Laurate, Sorbitan Sesquioleate, Sorbitan Oleate, Sorbitan Tristearate, Sorbitan Palmitate, and Sorbitan Trioleate. *J. Am. Coll. Toxicol*. 4:65–121.

Food and Drug Administration (FDA). 1984. Cosmetic product formulation and frequency of use data. *FDA database*. Washington, DC: FDA.

FDA. 1998. Frequency of use of cosmetic ingredients. *FDA database*. Washington, DC: FDA.

Galloway, S. M., D. A. Deasy, C. L. Bean, et al. 1987. Effects of high osmotic strength on chromosome aberrations, sister-chromatid exchanges and DNA strand breaks, and the relation to toxicity. *Mutat. Res*. 189:15–25.

Hill Top Research, Inc. 1988. Repeated insult patch test. Unpublished data submitted by CTFA, 6-26-98. (11 pages.)[2]

Ishidate, M., Jr., T. Sofuni, K. Yoshikawa, et al. 1984. Primary mutagenicity screening of food additives currently used in Japan. *Food Chem. Toxicol*. 22:623–636.

MacKenzie, K. M., W. N. Hauck, A. G. Wheeler, and F. J. C. Roe. 1986. Three-generation reproduction study of rats ingesting up to 10% sorbitol in the diet—and a brief review of the toxicological status of sorbitol. *Food Chem. Toxicol*. 24:191–200.

MB Research Labs. 1991. Chorioallantoic membrane vascular assay (CAMVA) on PEG-20 Sorbitan Beeswax. Unpublished data submitted by CTFA, 6-26-98. (6 pages.)[2]

Modderman, J. P. 1993. Safety assessment of hydrogenated starch hydrolysates. *Regul. Toxicol. Pharmacol*. 18:80–114.

National Testing Corporation. 1988. Eytex assay on PEG-20 Sorbitan Beeswax. Unpublished data submitted by CTFA, 6-26-98. (4 pages.)[2]

Nikitakis, J. M., and G. N. McEwen, Jr., eds. 1990. *CTFA compendium of cosmetic ingredient composition—Descriptions II*. Washington, DC: CTFA.

Roe, F. J. C. 1984. Perspectives in carbohydrate toxicology with special reference to carcinogenicity. *Swed. Dent. J*. 8:99–111.

Sato, S., T. Ohka, M. Nagao, K. Tsuji, and T. Kosuge. 1979. Reduction in mutagenicity of cigarette smoke condensate by added sugars. *Mutat. Res*. 60:155–161.

Unichema International. 1996. Polyalcohol Isostearate Esters: Safety Evaluation. Unpublished data submitted by CTFA, 5–98. (9 pages.)[2]

Wenninger, J. A., and G. N. McEwen, Jr., eds. 1997. *International cosmetic ingredient dictionary and handbook*, 7th ed., vol. 2, 990–991. Washington, DC: CTFA.

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