Final Report on the Safety Assessment of PEG-20 Sorbitan Cocoate; PEG-40 Sorbitan Diisostearate; PEG-2, -5, and -20 Sorbitan Isostearate; PEG-40 and -75 Sorbitan Lanolate; PEG-10, -40, -44, -75, and -80 Sorbitan Laurate; PEG-3, and -6 Sorbitan Oleate; PEG-80 Sorbitan Palmitate; PEG-40 Sorbitan Perisostearate; PEG-40 Sorbitan Peroleate; PEG-3, -6, -40, and -60 Sorbitan Stearate; PEG-20, -30, -40, and -60 Sorbitan Tetraoleate; PEG-60 Sorbitan Tetrastearate; PEG-20 and -160 Sorbitantriisostearate; PEG-18 Sorbitan Trioleate; PEG-40 and -50 Sorbitol Hexaoleate; PEG-30 Sorbitol Tetraoleate Laurate; and PEG-60 Sorbitol Tetrastearate—Addendum to the Final Report on the Safety Assessment of Polysorbates[1]

The PEGs Sorbitan/Sorbitol Fatty Acid Esters are ethoxylated sorbitan and sorbitol esters of fatty acids that function as surfactants in cosmetic formulations. PEG is the terminology used in the cosmetics industry for polyethylene glycol. Ingredients in a subset of this group are referred to by the cosmetics industry as Polysorbates and were previously reviewed by the Cosmetic Ingredient Review (CIR) Expert Panel. These ingredients are formed by the esterification of sorbitol or sorbitan with a fatty acid, followed by the chemical addition of ethylene oxide. 1,4-Dioxane and other water-soluble by-products may be formed. Most of the available safety test data relate to the Polysorbates or their components, Sorbitan Fatty Acids, PEGs, and Fatty Acids, which also have completed safety assessments. These ingredients are readily hydrolyzed by blood and pancreatic lipases, with the fatty acid moiety absorbed and metabolized as any dietary fatty acid and the PEG Sorbitan moiety excreted mainly in the urine. It is well recognized that PEGs are readily absorbed through damaged skin. Polysorbates have low toxicity in both acute and long-term toxicity studies using animals. Sorbitan Esters and PEGs also were relatively nontoxic to animals. Growth retardation and diarrhea in mice, microscopic changes of the urinary bladder, spleen, kidneys, and gastrointestinal tract in rats, and decreased body and organ weights, diarrhea, and hepatic lesions in rats were noted in subchronic feeding studies, whereas other studies found no effects. One chronic toxicity study using hamsters noted microscopic lesions of the urinary bladder, kidneys, spleen, and gastrointestinal tract, whereas other studies in monkeys, mice, rats, dogs, and hamsters were negative. The Polysorbates were nonirritating to mildly irritating in both in vivo and in vitro ocular irritation assays at concentrations ranging from 1% to 100%. In teratology studies of thalidomide, the PEG-20 Sorbitan Laurate vehicle (10 ml/kg) had no effect on the developing mouse embryo. In other studies, reproductive and developmental effects were seen primarily at exposure levels that were maternally toxic. It is recognized that the PEG monomer, ethylene glycol, and certain of its monoalkyl ethers are reproductive and developmental toxins. The CIR Expert Panel concluded that, as the PEGs Sorbitan and Sorbitol Esters are chemically different from the alkyl ethers of ethylene glycol and the alkyl ethers are not present as impurities, these ingredients pose no reproductive or developmental hazard. In subchronic and chronic oral toxicity studies, the PEGs did not cause adverse reproductive effects. The Polysorbates were nonmutagenic in a number of bacterial and mammalian systems. Data were available showing that treatment of cells in culture with Sorbitan Oleate reduces DNA repair following UV irradiation, but these data were not considered significant in view of the available carcinogenesis data. In general, the Polysorbates were not oral or dermal carcinogens. Data on the cocarcinogenesis of certain Sorbitan Esters were positive, but only with high exposure levels and a high frequency of exposure, and the results lacked a dose response.

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[1]Reviewed by the Cosmetic Ingredient Review Expert Panel. Rebecca S. Lanigan, former Scientific Analyst and Writer, prepared this report. The original safety assessment of Polysorbates was published in 1984. *J. Am. Col. Toxicol.* 3:1–82. Address correspondence to Director, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 310, Washington, DC 20036, USA.

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The Polysorbates also had antitumor activity in animal studies. The Polysorbates were nontoxic by the oral route in clinical studies, but a Polysorbate vehicle for a neonatal parenteral supplement caused the deaths of 38 premature infants. The Polysorbates had little potential for human skin irritation, sensitization, and phototoxicity in extensive clinical studies. Likewise, PEGs were nonsensitizers, but cases of systemic toxicity and contact dermatitis were observed in burn patients that were treated with PEG-based topical ointments. The Sorbitan Esters had the potential to cause cutaneous irritation in humans, and could cause sensitization in patients with damaged skin. Several of the Polysorbates enhanced skin penetration of other chemicals. Overall, these data were considered an adequate basis for assessing the safety of the entire group. The CIR Expert Panel concluded that these ingredients were safe for use in cosmetics at the levels in current use (not more than a 25% concentration) with the caveat that they should not be used on damaged skin.

INTRODUCTION

The PEGs Sorbitan Fatty Acid Esters are ethoxylated sorbitol and sorbitan esters of fatty acids that function as surfactants in cosmetic formulations. This assessment is an addendum to the review of the Polysorbates, which is the name given by the cosmetics industry to a series of specific chain length PEGs Sorbitan Fatty Acid Esters (Elder 1984). Table 1 presents the ingredients included in this safety assessment along with a list of those Polysorbates previously reviewed.

The Polysorbates are generally recognized as safe (GRAS) food additives and are used as emulsifiers in pharmaceutical products. Data on the Polysorbates, sorbitan esters, fatty acids, and Polyethylene glycols (PEGs), have been updated as a further basis for the assessment of safety of the additional PEGs Sorbitan and Sorbitol Fatty Acid Esters. The Cosmetic Ingredient Review (CIR) Expert Panel has concluded previously that:

Polysorbates 20, 21, 40, 60, 61, 65, 80, 81, and 85 are safe as cosmetic ingredients in the concentration of present use (Elder 1984).

Sorbitan Stearate, Sorbitan Laurate, Sorbitan Sesquioleate, Sorbitan Oleate, Sorbitan Tristearate, Sorbitan Palmitate, and Sorbitan Trioleate are considered safe as cosmetic ingredients under present conditions of concentration and use (Elder 1985).

Oleic, Lauric, Palmitic, Myristic, and Stearic Acids are safe in present practices of use and concentration in cosmetics (Elder 1987).

Isostearic Acid is safe as a cosmetic ingredient in the present practices of use (Elder 1983).

Coconut Oil, Coconut Acid, Hydrogenated Coconut Oil, and Hydrogenated Coconut Acid are safe for use as cosmetic ingredients (Elder 1986).

Lanolin and related Lanolin materials described herein are safe for topical application to humans in the present practices of use and concentration (Elder 1980).

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 the Final Report. The Expert Panel recommends that cosmetic formulations containing these PEGs not be used on damaged skin (Andersen 1993).

CHEMISTRY

Definition and Structure

The PEGs Sorbitan and Sorbitol Fatty Acid Esters (Table 2) are ethoxylated fatty acid esters of the hexahydric alcohol,

TABLE 1
Ingredient list

New ingredients (this report)	Polysorbate ingredients previously reviewed (Elder 1984)
PEG-20 Sorbitan Cocoate	
PEG-40 Sorbitan Diisostearate	PEG-4, -20 Sorbitan Laurate (*Polysorbates 21 and 20*)
PEG-2, -5, -20 Sorbitan Isostearate	
PEG-40, -75 Sorbitan Lanolate	
PEG-10, -40, -44, -75, -80 Sorbitan Laurate	
PEG-3, -6 Sorbitan Oleate	PEG-5, -20 Sorbitan Oleate (*Polysorbates 81 and 80*)
PEG-80 Sorbitan Palmitate	PEG-20 Sorbitan Palmitate (*Polysorbate 40*)
PEG-40 Sorbitan Perisostearate	
PEG-40 Sorbitan Peroleate	
PEG-3, -6, -40, -60 Sorbitan Stearate	PEG-4, -20 Sorbitan Stearate (*Polysorbates 61 and 60*)
PEG-20, -30, -40, -60 Sorbitan Tetraoleate	
PEG-60 Sorbitan Tetrastearate	
PEG-20, -160 Sorbitan Triisostearate	
PEG-18 Sorbitan Trioleate	PEG-20 Sorbitan Trioleate (*Polysorbate 85*)
	PEG-20 Sorbitan Tristerate (*Polysorbate 65*)
PEG-40, -50 Sorbitol Hexaoleate	
PEG-30 Sorbitol Tetraoleate Laurate	
PEG-60 Sorbitol Tetrastearate	

sorbitol, and its mono- and dianhydrides. These ingredients conform generally to the formulas in Figure 1 (Chi, Scocca, and Huang 1978; Nikitakis and McEwen 1990a; Radian Corporation 1991; Chemline 1996; Food and Drug Administration [FDA] 1996; Wenninger et al. 2000). Structures were not available for PEG-20 Sorbitan Cocoate, PEG-40 and -75 Sorbitan Lanolate, PEG-40 Sorbitan Perisostearate, and PEG-40 Sorbitan Peroleate.

A subset of this group of ingredients is the Polysorbate family. Even though a different name is used, these are also PEGs Sorbitan Fatty Acid mono- or triesters. This family includes PEG-4 and -20 Sorbitan Laurate, PEG-4 and -20 Sorbitan Stearate, PEG-5 and -20 Sorbitan Oleate, PEG-20 Sorbitan Palmitate, PEG-20 Sorbitan Tristearate, and PEG-20 Sorbitan Trioleate (Elder 1984).

Chemical and Physical Properties

PEG-40 Sorbitan Lanolate is an amber-colored, soft paste that is soluble in dioxane, carbon tetrachloride, and water at 65°C. The hydroxyl value is 130 to 155, the maximum acid value is 3.0, the saponification value is 20 to 30, and the maximum amount of moisture is 1.0% (Nikitakis and McEwen 1990b).

PEG-4 Sorbitan Laurate (Polysorbate 21) is dispersible in water and soluble in ethanol and corn oil. Its hydroxyl value is 220 to 255, the saponification value is 100 to 115, Sorbitan Laurate (Polysorbate 20) is soluble in water, ethanol, ethyl acetate, and the maximum acid value is 4.0. The maximum amount of water is 3.0%. PEG-10 and -20 Sorbitan Laurate are clear, yellow, unctuous liquids with mild odors. PEG-20 Sorbitan Laurate (Polysorbate 20) is soluble in water, ethanol, ethyl acetate and methanol. PEG-10 Sorbitan Laurate is soluble in water, acetone, ethyl acetate, and the lower alcohols. Both are insoluble in mineral oil. For PEG-10 Sorbitan Laurate, the saponification value is 66 to 76, and the hydroxyl value is 150 to 168. The maximum values for acid, sulfated ash, and water are 2.0%, 0.15%, and 3.0%, respectively. PEG-20 Sorbitan Laurate has a saponification value of 40 to 50, a hydroxyl value of 96 to 108, and a maximum acid value of 2.0. The maximum amounts of sulfated ash and water are 0.15% and 3.0%, respectively (Nikitakis and McEwen 1990a). When the PEGs Sorbitan Laurate were heated to decomposition, acrid, irritating fumes were released (Lewis 1993b). The hydrophile lipophile balance value (HLB) value of PEG-4 Sorbitan Laurate is 13.3 (Cosmetic Science & Technology On-line 1997).

PEG-5 Sorbitan Oleate (Polysorbate 81) is an amber, unctuous liquid which can gel. It has a faint odor and is dispersible in water, ether, and ethylene glycol. It is soluble in ethanol, methanol, ethyl acetate, mineral oil, and corn oil. The saponification value is 96 to 104, the hydroxy value is 135 to 145, and the maximum acid value is 2.0. The maximum amounts of sulfated ash and water are 0.15% and 3.0%, respectively. PEG-20 Sorbitan Oleate (Polysorbate 80) is a lemon to orange-colored, oily liquid with a faint, characteristic odor. It is very soluble in water, and produces an odorless and nearly colorless aqueous solution (Nikitakis and McEwen 1990a). PEG-20 Sorbitan Oleate is soluble in dimethyl sulfoxide, ethanol, methanol, cottonseed and corn oils, mineral oil, ethyl acetate, and toluene. It is insoluble in mineral oil (Nikitakis and McEwen 1990a; Radian Corporation 1991). The saponification value is 45 to 55, the hydroxyl value is 65 to 80, and the maximum acid value is 2.0. The maximum amounts of sulfated ash and water are, respectively, 0.15% and 3.0% (Nikitakis and McEwen 1990a). The molecular weight of PEG-20 Sorbitan Oleate is 1309.68 Da, the specific gravity is 1.1, and the density is 1.064 g/ml. PEG-20 Sorbitan Oleate is "incompatible" with strong alkalis and oxidizers. The flash point of this compound is >110°C, and it is considered "probably combustible." The viscosity is 270 to 430 centistokes, the refractive index is 1.4756 at 20°C, and the pH of a 5% aqueous solution is 5 to 7 (Radian Corporation 1991).

PEG-20 Sorbitan Palmitate (Polysorbate 40) is a clear yellow, unctuous liquid with a faint, characteristic odor. It is soluble in water, methanol, and ethanol, and is insoluble in mineral oil. The saponification value is 43 to 49, the hydroxyl value is 89 to 105, and the maximum acid value is 2.0. The maximum amounts of sulfated ash and water found in PEG-20 Sorbitan Palmitate, respectively, are 0.15% and 3.0% (Nikitakis and McEwen 1990a).

PEG-40 Sorbitan Peroleate is a viscous, oily, clear yellow liquid which has a faint characteristic odor. It is soluble in mineral or vegetable oil and is dispersible in water. The hydroxyl value of PEG-40 Sorbitan Peroleate is 20 to 38, the saponification value is 100 to 115, and the acid value is 8.0 to 12.0 (Nikitakis and McEwen 1990b).

PEG-4 Sorbitan Stearate (Polysorbate 61) is a tan, waxy solid at room temperature, and has a mild odor. It is soluble in methanol and ethanol, dispersible in distilled water, and insoluble in ethylene glycol and propylene glycol. The saponification value is 95 to 115, the hydroxy value is 170 to 200, and the maximum acid value is 2.0. The maximum amounts of sulfated ash and water are 0.25% and 3.0%, respectively. PEG-20 Stearate (Polysorbate 60) is a lemon yellow, oily liquid with a tendency to gel at room temperature. It is soluble in water, ethanol, and ethyl acetate, but is insoluble in mineral and vegetable oils. The saponification value is 45 to 55, the hydroxyl value is 81 to 96, and the maximum acid value is 2.0. The maximum amounts of sulfated ash and water are 0.25% and 3.0%, respectively (Nikitakis and McEwen 1990a).

PEG-20 Sorbitan Trioleate (Polysorbate 85) is a clear, amber, unctuous liquid that can gel, and has a characteristic odor. It is dispersible in water, and is soluble in most vegetable and mineral oils as well as in a variety of organic solvents. The saponification value is 82 to 95, the hydroxyl value is 39 to 52, and the maximum acid value is 2.0. The maximum amount of sulfated ash is 0.15% (Nikitakis and McEwen 1990a).

PEG-20 Sorbitan Tristearate (Polysorbate 65) is a tan, waxy solid with a faint, characteristic odor. It is soluble in ethanol, methanol, mineral oil, vegetable oil, acetone, and ether, and is dispersible in water. The saponification value is 88 to 98, the hydroxyl value is 44 to 60, and the maximum acid value is 2.0. The maximum amounts of sulfated ash and

TABLE 2
Information on PEGs Sorbitan/Sorbitol Fatty Acid Esters*

Ingredient	CAS number, definition and generic names	Calculated molecular weight[a]	Synonym(s)
PEG-20 Sorbitan Cocoate	Ethoxylated sorbitan ester of coconut acid with average of 20 moles ethylene oxide	>1043[b]	Polyethylene glycol 1000 sorbitan cocoate Polyoxyethylene (20) sorbitan cocoate
PEG-40 Sorbitan Diisostearate	Ethoxylated sorbitan diester of isostearic acid with average of 40 moles ethylene oxide	2456	Polyethylene glycol 1000 sorbitan diisostearate Polyoxyethylene (40) sorbitan diisostearate
PEG-2 Sorbitan Isostearate		518	Polyethylene glycol 100 sorbitan isostearate Polyoxyethylene (2) sorbitan isostearate
PEG-5 Sorbitan Isostearate	CAS No. 66794-58-9; ethoxylated sorbitan monoesters of isostearic acid with average of *n* moles ethylene oxide	650	Polyethylene glycol (5) sorbitan isostearate Polyoxyethylene (5) sorbitan isostearate
PEG-20 Sorbitan Isostearate		1310	Polyethylene glycol 1000 sorbitan monoisostearate Polyoxyethylene sorbitan isostearate (20 E.O.) Polyoxyethylene (20) sorbitan monoisostearate
PEG-40 Sorbitan Lanolate	CAS No. 8036-77-9; ethoxylated sorbitan derivatives of lanolin acid (q.v.) with average of *n* moles ethylene oxide	>2807[b]	Polyethylene glycol 2000 sorbitan lanolate Polyoxyethylene (40) sorbitol lanolate Polyoxyethylene sorbitol lanolate (40 E.O.)
PEG-75 Sorbitan Lanolate	CAS No. 8051-13-6; ethoxylated sorbitan derivatives of lanolin acid (q.v.) with average of *n* moles ethylene oxide	>2807[b]	Polyethylene glycol 4000 sorbitan lanolate Polyoxyethylene (75) sorbitol lanolate
PEG-10 Sorbitan Laurate	CAS No. 9005-64-5; mixtures of laurate partial esters of the hexahydric alcohol, sorbitol and its anhydrides, condensed with average of *n* moles ethylene oxide for each mole of sorbitol and sorbitol mono- and dianhydrides	788	Polyethylene glycol 500 sorbitan monolaurate
PEG-40 Sorbitan Laurate		2108	Polyethylene glycol 2000 sorbitan monolaurate
PEG-44 Sorbitan Laurate		2284	
PEG-75 Sorbitan Laurate	Generic names include sorbitan, monododecanoate, poly(oxy-1,2-ethanediyl) derivative	3648	Polyethylene glycol 4000 sorbitan monolaurate
PEG-80 Sorbitan Laurate		3868	
PEG-3 Sorbitan Oleate	CAS No. 9005-65-6; ethoxylated sorbitan esters of oleic acid with average of *n* moles ethylene oxide	560	Polyethylene glycol (3) sorbitan monooleate Polyoxyethylene (3) sorbitan monooleate
PEG-6 Sorbitan Oleate	Generic names include: sorbitan mono-9-octadecanoate poly(oxy-1,2-ethanediyl) derivative; polyethylene oxide sorbitan mono-oleate; sorbitan mono-oleate polyoxyethylene; sorbitan, monooleate polyoxyethylene derivative; and ethosorbitan monooleate	692	Polyethylene glycol 300 sorbitan monooleate Polyoxyethylene sorbitan monooleate (6 E.O.)

PEG-80 Sorbitan Palmitate	CAS No. 9005-66-7; ethoxylated sorbitan monoester of palmitic acid with average 80 moles ethylene oxide	3922	Polyethylene glycol (80)sorbitan monopalmitate Polyoxyethylene (80) sorbitan monopalmitate
PEG-40 Sorbitan Perisostearate	Mixture of isostearic acid esters of sorbitol condensed with average of 40 moles ethylene oxide	>2807[b]	Polyethylene glycol 2000 sorbitan perisostearate Polyoxyethylene (40) sorbitan perisostearate
PEG-40 Sorbitan Peroleate	Mixture of oleic acid esters of sorbitol condensed with average of 40 moles ethylene oxide	>2807[b]	Polyethylene glycol 2000 sorbitan peroleate Polyoxyethylene (40) sorbitan peroleate
PEG-3 Sorbitan Stearate	CAS No. 9005-67-8; ethoxylated sorbitan monoesters of stearic acid with average of *n* moles ethylene oxide	562	Polyethylene glycol (3) sorbitan monostearate Polyoxyethylene (3) sorbitan monostearate
PEG-6 Sorbitan Stearate		685	Polyethylene glycol 300 sorbitan monostearate Polyoxyethylene (6) sorbitan monostearate Polyoxyethylene sorbitan monostearate (6 E.O.)
PEG-40 Sorbitan Stearate		2190	Polyethylene glycol 2000 sorbitan stearate Polyoxyethylene (40) sorbitan stearate
PEG-60 Sorbitan Stearate		3070	Polyethylene glycol 3000 sorbitan monostearate Polyoxyethylene (60) sorbitan monostearate
PEG-20 Sorbitan Tetraoleate	Tetraesters of oleic acid and polyethylene glycol ether of sorbitol, with average of *n* moles ethylene oxide	2100	
PEG-30 Sorbitan Tetraoleate		2540	Polyethylene glycol (30) sorbitan tetraoleate Polyoxyethylene (30) sorbitan tetraoleate
PEG-40 Sorbitan Tetraoleate		2980	Polyethylene glycol 2000 sorbitan tetraoleate *Polyoxyethylene (40) sorbitan tetraoleate*
PEG-60 Sorbitan Tetraoleate		4772	Polyethylene glycol 3000 sorbitan tetraoleate Polyoxyethylene (60) sorbitan tetraoleate
PEG-60 Sorbitan Tetrastearate	Tetraester of stearic acid and polyethylene glycol ether of sorbitol, with average of 60 moles ethylene oxide	4780	Polyethylene glycol (60) sorbitan tetrastearate Polyoxyethylene (60) sorbitan tetrastearate
PEG-20 Sorbitan Triisostearate	Triesters of isostearic acid and polyethylene glycol of sorbitol, with average of *n* moles ethylene oxide	1842	Polyethylene glycol 1000 sorbitan triisostearate Polyoxyethylene (20) sorbitan triisostearate
PEG-160 Sorbitan Triisostearate		64622	
PEG-18 Sorbitan Trioleate	Ethoxylated sorbitan triester of oleic acid with average of 18 moles ethylene oxide	1748	
PEG-40 Sorbitol Hexaoleate	Oleic acid hexaesters of ethoxylated sorbitol with average of *n* moles ethylene oxide	3525	Polyethylene glycol 2000 sorbitol hexaoleate Polyoxyethylene (40) sorbitol hexaoleate
PEG-50 Sorbitol Hexaoleate		3965	Polyethylene glycol (50) sorbitol hexaoleate Polyoxyethylene (50) sorbitol hexaoleate
PEG-30 Sorbitol Tetraoleate Laurate	Oleic acid tetraester and lauric acid ester of ethoxylated sorbitol with average of 30 moles ethylene oxide	2505	Polyethylene glycol (30) sorbitol tetraoleate laurate Polyoxyethylene (30) sorbitol tetraoleate laurate
PEG-60 Sorbitol Tetrastearate	Stearic acid tetraester of ethoxylated sorbitol with average of 60 moles ethylene oxide	3428	Polyethylene glycol (60) sorbitol tetrastearate Polyoxyethylene (60) sorbitol tetrastearate Polyoxyethylene sorbitol tetrastearate (60 E.O.)

*Chi, Scocca, and Huang 1978; Nikitakis and McEwen, 1990a; Radian Corporation 1991; Chemline 1996; FDA, 1996; Wenninger et al. 2000.

[a]Molecular weight calculated using $(W + X + Y + Z) = n$ or $(U + V + W + X + Y + Z) = n$, where n equals the average moles of ethylene oxide (the number in the name).

[b]If structures were unavailable, the value is the sum of the molecular weights of the sorbitan and PEG moieties only.

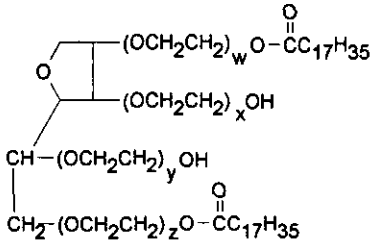
water are 0.25% and 3.0%, respectively (Nikitakis and McEwen 1990a).

Method of Manufacture

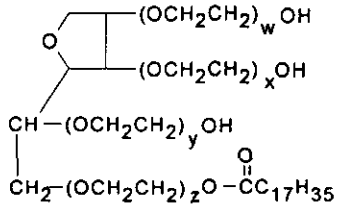
The Polysorbates, including PEG-4 and -20 Sorbitan Laurate, are prepared from sorbitol and sorbitol anhydrides (Figure 2) by the elimination of sorbitan, which is then partially esterifiled with a fatty acid. The resulting hexitan ester is polymerized with ethylene oxide (Gennaro 1990) and steam-stripped to remove water-soluble impurities such as 1,4-dioxane (Elder 1984; Smolinske 1992).

PEG-20 Sorbitan Palmitate (Polysorbate 40) is prepared by the reaction of ethylene oxide with sorbitan monopalmitate, by the addition of polyoxyethylene chains to the nonesterifed hydroxyls, and by the esterification of sorbitol with one or three molecules of fatty acid. PEG-20 Sorbitan Oleate (Polysorbate 80) is manufactured by the partial esterification of sorbitan with fatty acid to yield a hexitan ester or by the chemical addition of ethylene oxide to yield the polyoxyethylene derivative. In addition, it can be formed by the elimination of water from sorbitol to form sorbitan, the cyclic sorbitol anhydride (Hazardous Substances Database [HSDB] 1996).

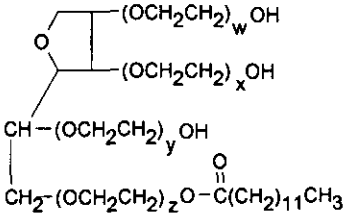
PEG-40 Sorbitan Diisostearate



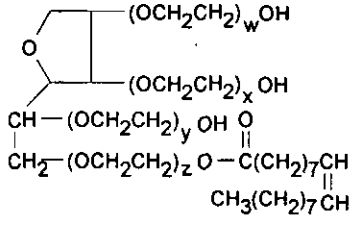
PEG-n Sorbitan Isostearate



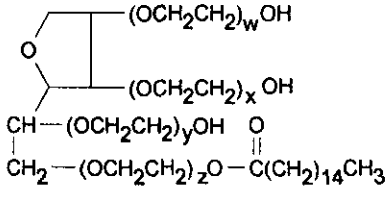
PEG-n Sorbitan Laurate



PEG-n Sorbitan Oleate



PEG-n Sorbitan Palmitate



PEG-n Sorbitan Stearate

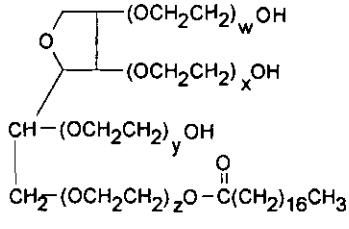


FIGURE 1

Formulas for selected PEGs Sorbitan/Sorbitol Fatty Acid Esters. The naming convention has the value of "*n*" in the ingredient name as sum of U + V + W + X + Y + Z. Chi, Scocca, and Huang 1978; Nikitakis and McEwen 1990a; Radian Corporation 1991; Chemline 1996; FDA 1996; Wenninger et al. 2000. *(Continued)*

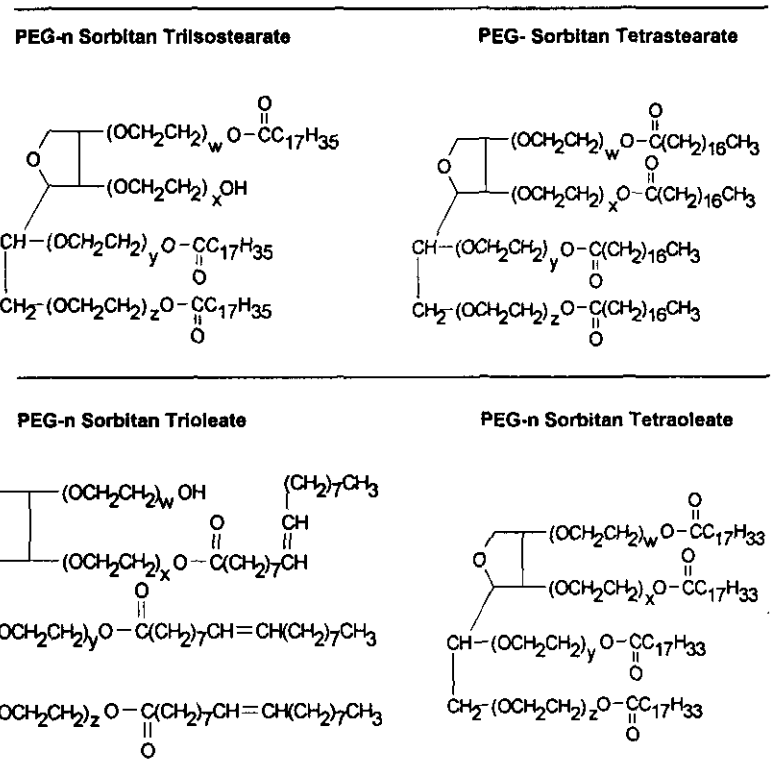


FIGURE 1
(Continued)

Impurities

Impurities in the Polysorbates include peroxides, isosorbide ethoxylates, free fatty acids, lead, and arsenic (Elder 1984; Smolinske 1992).

Each of the Polysorbates can have a complex fatty acid moiety, as the fatty acids used in the production of cosmetic ingredients frequently contain fatty acids other than the principal acid named. When the fatty acid moiety compositions of the Polysorbates were determined, Polysorbate 20 (PEG-20 Sorbitan Laurate) was comprised of 36.9% lauric acid, 15.3% palmitic acid, 13.7% oleic acid, and 22.8% myristic acid. Polysorbate 40 (PEG-20 Sorbitan Palmitate) consisted of 86.4% palmitic acid and 10.2% stearic acid. Polysorbate 60 (PEG-20 Sorbitan Stearate) was comprised of 44.4% palmitic acid and 45.0% stearic acid. Polysorbate 80 (PEG-20 Sorbitan Oleate) consisted of 6.4% palmitic acid, 76.9% oleic acid, and 6.4% palmitoleic acid (Elder 1984).

1,4-Dioxane was detected at concentrations of 5.5 to 378 ppm in samples of PEG-20 Sorbitan Stearate and PEG-20 Sorbitan Oleate; however, the Polysorbates are steam-stripped during the manufacturing process to remove water-soluble by-products such as 1,4-dioxane (Elder 1984).

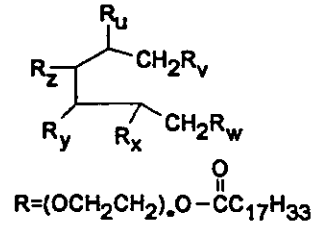
The sorbitan esters could contain some residual free acid and alcohol. Minor impurities include arsenic (not more than 3 ppm), lead (not more than 10 ppm), and water (Elder 1985).

PEG-6 could contain small amounts of monomer and dimers. The amounts were not 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 μEq 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).

Reactivity

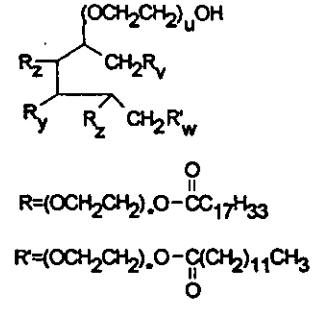
The Polysorbates interacted with specific drugs in biopharmaceutical and drug release studies. They inactivated preservatives and altered the activities of cationic germicidal agents in numerous cosmetic ingredients, including *p*-hydroxybenzoic acid and its methyl, ethyl, propyl, and butyl esters; benzoic acid; benzyl alcohol; capric acid; chlorbutol; chlorocresol; sorbic acid; and glyceryl monolaurate (Elder 1984).

PEG-n Sorbitol Hexaoleate



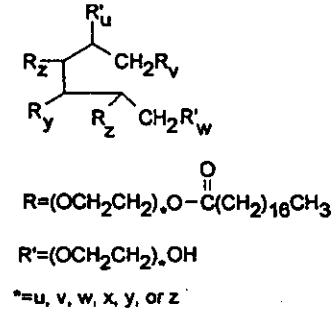
The * in the R group is either u, v, w, x, y, or z

PEG-30 Sorbitol Tetraoleate Laurate



*=v, w, x, y, or z

PEG-60 Sorbitol Tetrastearate



*=u, v, w, x, y, or z

FIGURE 1
(Continued)

Aqueous solutions of PEG-20 Sorbitan Laurate undergo autoxidation during storage. During autoxidation, the peroxide number increases and subsequently decreases, the acidity increases continuously, pH and surface tension fall and plateau, and the cloud point drops such that turbidity begins at room temperature. Autoxidation is accelerated by light, increased temperature, and a copper sulfate catalyst. Hydrolysis also occurs, releasing lauric acid. Hydrolysis has major effects at room temperature, and the oxyethylene units undergo chain shortening at temperatures above 40°C (Donbrow, Azaz, and Pillersdorf 1978).

Degradation of PEG-20 Sorbitan Laurate, PEG-20 Sorbitan Palmitate, and PEG-20 Sorbitan Stearate has been detected in unopened commercial samples of the Polysorbates, and can occur in cosmetic formulations due to the action of microorganisms. Bacteria in deionized water used to manufacture cosmetics were found to enzymatically decompose PEG-20 Sorbitan Laurate (Elder 1984).

Analytical Methods

PEG-10 Sorbitan Laurate has been determined using infrared spectrometry and nuclear magnetic resonance (Nikitakis and McEwen 1990a). PEG-40 Sorbitan Peroleate has been analyzed by infrared spectrometry (Nikitakis and McEwen 1990b). Polyoxyethylene esters are characterized by a very strong infrared absorption peak at 9 μm (Elder 1984).

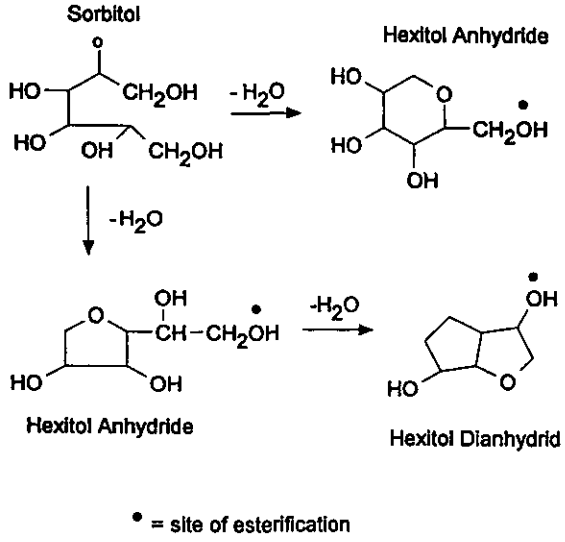


FIGURE 2
Mechanisms of Hexitol Anhydride Derivation (Canterbery 1997).

Ultraviolet Absorption

The maximum absorbances of 0.1504 mg/ml of aqueous PEG-20 Sorbitan Laurate were 0.100 at 245 nm and approximately 0.140 at 320 nm (λ_{max}). PEG-20 Sorbitan Laurate did not absorb ultraviolet (UV) radiation above 365 nm (National Toxicology Program [NTP], 1992a).

Sorbitans Laurate, Sesquioleate, Palmitate, and Trioleate did not absorb UVA or UVB radiation in a UV spectral analysis (Elder 1985).

USE

Cosmetic

The PEGs Sorbitan Fatty Acid Esters are surfactants and function as emulsifying agents, cleansing agents, and solubilizing agents in cosmetic formulations (Wenninger et al. 2000). Current uses of ingredients in this report are presented in Table 3. In 1998, PEG-20 Sorbitan Isostearate, PEG-40 Sorbitan Lanolate, PEG-10, -44, and -80 Sorbitan Laurate, PEG-40 Sorbitan Peroleate, PEG-20 and -40 Sorbitan Tetraoleate, and PEG-18 Sorbitan Trioleate were used in a total of 81 formulations. The Polysorbates were used in 1418 formulations. No uses were reported for the remaining PEGs Sorbitan Fatty Acid Esters (Food and Drug Administration [FDA] 1998).

Data submitted by industry indicated that PEG-60 Sorbitan Tetratoleate, PEG-40 Sorbitan Tetraoleate, and PEG-160 Sorbitan Triisostearate are used in cosmetics at concentrations of 0.5% to 10% (Cosmetic, Toiletry, and Fragrance Association [CTFA] 1998a). In 1984, PEG-10 Sorbitan Laurate was used at concentrations ≤10%. Concentrations of 1% to 5% PEG-44 and -75 Sorbitan Laurate were used in cosmetic formulations. The concentration of use of PEG-40 Sorbitan Peroleate was typically 0.1% to 1%, but concentrations of 1% to 5% and 10% to 25% were reported in some categories. PEG-20 Sorbitan Isostearate was used at concentrations of 1% to 10%, PEG-40 Sorbitan Lanolate was used at 0.1% to 1%, and PEG-75 Sorbitan Lanolate was used at concentrations up to 10%. The Polysorbates were generally used at concentrations up to 5%, but a small number of formulations contained these ingredients at concentrations greater than 50% (FDA 1984).

Noncosmetic

PEG-20 Sorbitan Oleate (Polysorbate 80; USP grade) is used as an emulsifier and dispersing agent for medicinal products designed for internal use, and is a pharmaceutic aid (surfactant). It is a defoamer and emulsifier in foods, and a neutralizer for quaternary ammonium compounds in disinfectants. PEG-20 Sorbitan Palmitate is used as an emulsifier and dispersing agent, flavoring agent, textile finish, surfactant, and foaming and defoaming agent, and is used in pharmaceuticals, shortenings, and baked goods. In veterinary medicine, PEG-20 Sorbitan Palmitate is used in feeds (as emulsifiers), milk replacers, pesticides, vitamins, parenterals, vaccines, oral pharmaceuticals, and intramammary and assorted topical treatments (Radian Corporation 1991; HSDB 1996).

GENERAL BIOLOGY

Absorption, Metabolism, Distribution, and Excretion

Polysorbates

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. When fed to rats at a dietary concentration of 10%, the efficiencies at which the radiolabelled fatty acid portions of PEG-20 Sorbitan Oleate, PEG-20 Sorbitan Stearate, and PEG-20 Sorbitan Tristearate were hydrolyzed and absorbed were 100%, 98%, and 84%, respectively. 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 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 after administration of PEG-20 Sorbitan Oleate. None was detected in expired CO_2, liver, kidneys, spleen, adrenal glands, brain, gonads, or fat. Similar results were observed following intravenous injection of PEG-20 Sorbitan Laurate. In a study in which 4.5 g/day of PEG-20 Sorbitan Oleate was fed to rats, approximately 95% of the polyoxyethylene fraction was excreted in the feces and 5% was excreted in the urine. No polyoxyethylated fatty acids

TABLE 3
Product formulation data (FDA 1998)

Product category	Total no. of formulations in category	Total no. containing ingredient
PEG-20 Sorbitan Isostearate		
Cuticle softeners	19	1
Moisturizing preparations	769	1
1998 total for PEG-20 Sorbitan Isostearate		**2**
PEG-40 Sorbitan Lanolate		
Hair conditioners	636	1
Tonics, dressings, and other hair grooming aids	549	1
Other hair preparations	276	3
Hair lighteners with color	6	1
Other hair coloring preparations	59	1
1998 total for PEG-40 Sorbitan Lanolate		**7**
PEG-4 Sorbitan Laurate (Polysorbate 21)		
Other fragrance preparations	148	1
Moisturizing preparations	769	1
Suntan gels, creams, and liquids	136	2
1998 total for PEG-4 Sorbitan Laurate		**4**
PEG-10 Sorbitan Laurate		
Shampoos (noncoloring)	860	1
Nail polish and enamel removers	34	1
1998 total for PEG-10 Sorbitan Laurate		**2**
PEG-20 Sorbitan Laurate (Polysorbate 20)		
Baby shampoos	21	1
Other baby products	29	2
Bath oils, tablets, and salts	124	2
Bubble baths	200	11
Other bath preparations	159	14
Eyeliner	514	10
Eye lotion	18	4
Eye makeup remover	84	11
Mascara	167	3
Other eye makeup preparations	120	11
Colognes and toilet waters	656	5
Perfumes	195	8
Powders	247	43
Other fragrance preparations	148	4
Hair conditioners	636	40
Hair sprays (aerosol fixatives)	261	14
Permanent waves	192	6
Rinses (noncoloring)	40	2
Shampoos (noncoloring)	860	48
Tonics, dressings and other hair grooming aids	549	61
Wave sets	55	8
Other hair preparations	276	25
Hair dyes and colors	1572	44
Hair rinses (coloring)	33	1
Hair color sprays (aerosols)	4	1
Hair bleaches	113	2

TABLE 3
Product formulation data (FDA 1998) *(Continued)*

Product category	Total no. of formulations in category	Total no. containing ingredient
PEG-20 Sorbitan Laurate (Polysorbate 20)-continued		
Other hair coloring preparations	59	2
Blushers (all types)	238	4
Foundations	287	21
Lipstick	790	2
Makeup bases	132	29
Makeup fixatives	11	3
Other makeup preparations	135	3
Cuticle softeners	19	1
Nail creams and lotions	17	2
Nail polish and enamel	80	1
Other manicuring preparations	61	2
Dentifrices	38	3
Mouthwashes and breath fresheners	49	7
Bath soaps and detergents	385	16
Deodorants (underarm)	250	3
Douches	5	1
Other personal cleanliness products	291	10
Aftershave lotion	216	9
Shaving cream	139	13
Other shaving preparation products	60	3
Cleansing preparations	653	58
Face and neck preparations (excluding shaving)	263	10
Body and hand preparations (excluding shaving)	796	39
Foot powders and sprays	35	1
Moisturizing preparations	769	33
Night preparations	188	5
Paste masks (mud packs)	255	11
Skin fresheners	184	38
Other skin care preparations	692	44
Suntan gels, creams, and liquids	136	6
Indoor tanning preparations	62	5
Other suntan preparations	38	4
1998 total for PEG-20 Sorbitan Laurate (Polysorbate 20)		**770**
PEG-44 Sorbitan Laurate		
Cleansing preparations	653	2
Skin fresheners	184	1
Other skin care preparations	692	5
1998 total for PEG-44 Sorbitan Laurate		**8**
PEG-80 Sorbitan Laurate		
Baby shampoos	21	10
Other baby products	29	5
Hair conditioners	636	1
Rinses (noncoloring)	40	1
Shampoos (noncoloring)	860	7
Other hair preparations	276	1
Bath soaps and detergents	385	2
Cleansing preparations	653	7
1998 total for PEG-80 Sorbitan Laurate		**34**

(Continued on next page)

TABLE 3
Product formulation data (FDA 1998) *(Continued)*

Product category	Total no. of formulations in category	Total no. containing ingredient
PEG-5 Sorbitan Oleate (Polysorbate 81)		
Other hair preparations	276	1
Blushers (all types)	238	2
Moisturizing preparations	769	1
1998 total for PEG-5 Sorbitan Oleate (Polysorbate 81)		**4**
PEG-20 Sorbitan Oleate (Polysorbate 80)		
Baby shampoos	21	2
Baby lotions, oils, powders, and creams	53	2
Bath oils, tablets, and salts	124	1
Bubble baths	200	5
Other bath preparations	159	4
Eyeliner	514	10
Eye makeup remover	84	1
Mascara	167	2
Other eye makeup preparations	120	3
Colognes and toilet waters	656	5
Powders	247	6
Other fragrance preparations	148	3
Hair conditioners	636	32
Hair sprays (aerosol fixatives)	261	16
Hair straighteners	63	1
Permanent waves	192	1
Shampoos (noncoloring)	860	9
Tonics, dressings, and other hair-grooming aids	549	12
Wave sets	55	2
Other hair preparations	276	5
Hair dyes and colors	1572	9
Hair shampoos (coloring)	24	1
Blushers (all types)	238	2
Face powders	250	1
Lipstick	790	1
Makeup bases	132	1
Rouges	12	1
Other makeup preparations	135	3
Nail creams and lotions	17	1
Nail polish and enamel removers	34	1
Dentifrices	38	1
Mouthwashes and breath fresheners	49	13
Bath soaps and detergents	385	2
Other personal cleanliness products	291	2
Aftershave lotion	216	3
Shaving cream	139	3
Other shaving preparation products	60	2
Cleansing preparations	653	4
Face and neck preparations (excluding shaving)	263	3
Body and hand preparations (excluding shaving)	796	10
Moisturizing preparations	769	27
Night preparations	188	4
Paste masks (mud packs)	255	4
Skin fresheners	184	6

TABLE 3
Product formulation data (FDA 1998) *(Continued)*

Product category	Total no. of formulations in category	Total no. containing ingredient
PEG-20 Sorbitan Oleate (Polysorbate 80)-continued		
Other skin care preparations	692	3
Indoor tanning preparations	62	1
1998 total for PEG-20 Sorbitan Oleate (Polysorbate 80)		**231**
PEG-20 Sorbitan Palmitate (Polysorbate 40)		
Other eye makeup preparations	120	1
Other fragrance preparations	148	4
Hair straightners	63	1
Tonics, dressings, and other hair-grooming aids	549	1
Other manicuring preparations	61	1
Cleansing preparations	653	7
Body and hand preparations (excluding shaving)	796	3
Moisturizing preparations	769	6
Night preparations	188	1
Other skin care preparations	692	6
Indoor tranning preparations	62	1
1998 total for PEG-20 Sorbitan Palmitate (Polysorbate 40)		**32**
PEG-40 Sorbitan Peroleate		
Bath oils, tablets, and salts	124	5
Powders	247	1
Other fragrance preparations	148	1
Face and neck preparations (excluding shaving)	263	1
Moisturizing preparations	769	5
1998 total for PEG-40 Sorbitan Peroleate		**13**
PEG-4 Sorbitan Stearate (Polysorbate 61)		
Baby lotions, oils, powders, and creams	53	3
Other baby products	29	1
Body and hand preparations (excluding shaving)	796	4
1998 total for PEG-4 Sorbitan Stearate (Polysorbate 61)		**8**
PEG-20 Sorbitan Stearate (Polysorbate 60)		
Baby lotions, oils, powders, and creams	53	3
Eyebrow pencil	91	14
Eyeliner	514	4
Eye shadow	506	2
Eye lotion	18	2
Mascara	167	7
Other eye makeup preparations	120	6
Other fragrance preparations	148	4
Hair conditioners	636	6
Hair straighteners	63	4
Tonics, dressings, and other hair-grooming aids	549	7
Other hair preparations	276	2
Other hair-coloring preparations	59	1
Blushers (all types)	238	1

(Continued on next page)

TABLE 3
Product formulation data (FDA 1998) *(Continued)*

Product category	Total no. of formulations in category	Total no. containing ingredient
PEG-20 Sorbitan Stearate (Polysorbate 60)-continued		
Foundations	287	30
Makeup bases	132	1
Other makeup preparations	135	4
Cuticle softeners	19	3
Nail creams and lotions	17	1
Other manicuring preparations	61	1
Aftershave lotion	216	1
Shaving cream	139	21
Cleansing preparations	653	29
Face and neck preparations (excluding shaving)	263	15
Body and hand preparations (excluding shaving)	796	41
Foot powders and sprays	35	3
Moisturizing preparations	769	58
Night preparations	188	9
Paste masks (mud packs)	255	13
Skin fresheners	184	2
Other skin care preparations	692	24
Suntan gels, creams, and liquids	136	2
Indoor tanning preparations	62	9
Other suntan preparations	38	2
1998 total for PEG-20 Sorbitan Stearate (Polysorbate 60)		**332**
PEG-40 Sorbitan Stearate		
Eye makeup remover	84	1
1998 total for PEG-40 Sorbitan Stearate		**1**
PEG-20 Sorbitan Tetraoleate		
Moisturizing preparations	769	3
1998 total for PEG-20 Sorbitan Tetraoleate		**3**
PEG-40 Sorbitan Tetraoleate		
Body and hand preparations (excluding shaving)	796	1
1998 total for PEG-40 Sorbitan Tetraoleate		**1**
PEG-18 Sorbitan Trioleate		
Other shaving preparations products	60	1
Cleansing preparations	653	3
Moisturizing preparations	769	1
Other skin care preparations	692	5
1998 total for PEG-18 Sorbitan Trioleate		**10**
PEG-20 Sorbitan Trioleate (Polysorbate 85)		
Eyeliner	514	2
Eye shadow	506	2
Eye makeup remover	84	1
Hair conditioners	636	1
Tonics, dressings, and other hair-grooming aids	549	3
Hair lighteners with color	6	1
Foundations	287	1
Makeup bases	132	1
Makeup fixatives	11	2

TABLE 3
Product formulation data (FDA 1998) *(Continued)*

Product category	Total no. of formulations in category	Total no. containing ingredient
PEG-20 Sorbitan Trioleate (Polysorbate 85)-continued		
Other makeup preparations	135	3
Cleansing preparations	653	4
Face and neck preparations (excluding shaving)	263	1
Body and hand preparations (excluding shaving)	796	3
Moisturizing preparations	769	3
Other skin care preparations	692	1
Suntan gels, creams, and liquids	136	1
Indoor tanning preparations	62	5
1998 total for PEG-20 Sorbitan Trioleate (Polysorbate 85)		**35**
PEG-20 Sorbitan Tristearate (Polysorbate 65)		
Hair conditioners	636	1
Cleasing preparations	653	1
1998 total for PEG-20 Sorbitan Tristearate (Polysorbate 65)		**2**

were detected in the urine, hence the polyoxyethylene moiety in the urine represented PEG Sorbitan, and not the parent ester. PEG-20 Sorbitan Oleate was most likely hydrolyzed by pancreatic lipase, with the liberated oleic acid following the normal metabolic pathways of unsaturated fatty acids (Elder 1984).

In an in vitro study on surfactant-induced alterations of permeability of rabbit oral mucosa, PEG-20 Sorbitan Oleate caused a lesser increase in permeability than other surfactants, including sodium lauryl sulfate (Siegel and Gordon 1986). The lingual frena was removed from anesthetized adult male New Zealand white rabbits. Mucosal "pieces" were mounted in modified Ussing chambers, which were filled with Krebs-Ringer phosphate solution bubbled with 100% oxygen. The half-chamber facing outside (oral side) of the tissue was filled with oxygenated phosphate solution, one of eight solutes, and the test substance. PEG-20 Sorbitan Oleate only caused significant increases in permeability at the greatest concentration tested (1.0%), and only to three of the eight solutes used (heptanediol, sucrose, inulin). Similar results were reported in an earlier study using canine oral mucosa (Siegel and Gordon 1985).

Sorbitan Esters

When ingested, Sorbitan Stearate was hydrolyzed to stearic acid and anhydrides of sorbitol. Approximately 90% of Sorbitan Stearate was absorbed and hydrolyzed when fed to rats in oil solution, and ~50% was absorbed and hydrolyzed when fed as a water emulsion. The ingredient did not accumulate in the fat stores of the rat body (Elder 1985).

Polyethylene Glycol

Gastrointestinal 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 intravenous 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).

Cytotoxic Effects

Two in vitro cutaneous toxicity assays used skin of male New Zealand white rabbits (2 cm^2 discs), from which the subcutaneous fat had been removed, to determine the cytotoxicity of various chemicals, including PEG-20 Sorbitan Laurate (van de Sandt, Rutten, and Koëter 1993). Concentrations tested ranged from 30% to 100% (w/v), and the test compounds were applied for 4 hours. At 24 and 48 hours mitochondrial activity was assessed by measuring the reduction of the tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to a MTT-formazan precipitate by mitochondrial dehydrogenases. Membrane damage was assessed by measuring the uptake of neutral red (NR), a vital dye that normally accumulates in the lysosomes of viable cells. Cytotoxicity was measured and expressed as the concentration that resulted in a 70% reduction in either MTT conversion (MTT-70) or NR uptake (NR-70), compared to the vehicle control, deionized water.

Topical treatment with PEG-20 Sorbitan Laurate inhibited MTT reduction in the cultured skin cells. PEG-20 Sorbitan Laurate reduced the amount of the NR that accumulated in the treated cell lysosomes. PEG-20 Sorbitan Laurate inhibited the accumulation of the dye, indicating that membrane damage had occurred. The MTT-70 was 18.0% and the NR-70 (extrapolated)

TABLE 4
Cytotoxic end points of PEG-20 Sorbitan Stearate, PEG-20 Sorbitan Oleate and PEG-20 Sorbitan Palmitate (Borenfreund and Shopsis 1985)

Cytotoxicity assay Cell line	NR-90[a] Murine Balb/c 3T3 fibroblasts	Highest tolerated dose			UI-50[b]
		Murine Balb/c 3T3 fibroblasts	HepG2 hepatoma cells	RAW 264-7 macrophages	Murine Balb/c 3T3 fibroblasts
PEG-20 Sorbitan Oleate	—	280 μg/ml	300 μg/ml	300 μg/ml	400 μg/ml
PEG-20 Sorbitan Palmitate	—	150 μg/ml	200 μg/ml	200 μg/ml	190 μg/ml
PEG-20 Sorbitan Stearate	0.160 μg/ml	220 μg/ml	250 μg/ml	220 μg/ml	230 μg/ml

[a]Concentration that resulted in 90% reduction of neutral red uptake.
[b]Concentration that induced 50% inhibition in [^{3}H] uridine uptake.

was 2.2%. The reductions in MTT conversion and NR uptake were dose-dependent.

In another NR assay (using human lymphocytes) for cytotoxicity, the LC_{50} for PEG-20 Sorbitan Stearate was 210 μg/ml (Arechabala et al. 1995). The cytotoxic endpoints were also determined for PEG-20 Sorbitan Stearate (Borenfreund and Shopsis 1985), PEG-20 Sorbitan Palmitate, and PEG-20 Sorbitan Oleate (Table 4).

The greatest tolerated doses of three Polysorbates to Balb/c murine fibroblasts (3T3 cells), human hepatoma cells ($HepG_2$), and murine macrophage cells (RAW 264.7) were 150 to 200 μg/ml for PEG-20 Sorbitan Palmitate, 220 to 250 μg/ml for PEG-20 Sorbitan Stearate, and 280 to 300 μg/ml for PEG-20 Sorbitan Oleate (Borenfreund and Borrero 1984).

A number of other cytotoxic assays to determine ocular or dermal irritation or antitumor activity has been performed on the Polysorbates. These studies are summarized in their respective sections in this report.

Immunologic Effects

Barnett and Bryant (1980) investigated the immunosuppressive effects of adjuvants—retinol and PEG-20 Sorbitan Oleate—after exposure to the antigen ovalbumin. To determine immunosuppressive effects, Balb/c mice were treated with adjuvant by intraperitoneal (IP) injection on day −1 and immunized with the antigen intravenously on days 1 and 30. The ovalbumin was injected alone, diluted with saline (0.1 μg/0.1 ml), or adsorbed onto 0.1 mg $Al(OH)_3$ (0.1-ml dose). The effects of the adjuvants on the humoral response were then quantified using the passive hemagglutination assay (PHA) and passive cutaneous anaphylaxis (PCA) assay. The PHA determined titers of specific IgM and IgG antibodies to ovalbumin, and used human type O^+ erythrocytes which were sensitized with chromic chloride and diluted to 0.5% in bovine serum albumin and saline. The 2-hour PCA assay was used to determine IgG_1 titers after immunization with ovalbumin alone. The other assay involved the intradermal injection of 0.05 ml of immune serum at the dorsal surface, followed by an intravenous injection of 500 μg antigen in 0.4% Evans blue dye. A positive reaction was indicated by a blue skin reaction of 5 mm or greater.

Control mice retreated with saline alone produced "substantial amounts" of IgG and IgM after immunization with the adsorbed antigen (Table 5). A similar response was observed after injection of ovalbumin:saline. In the other control group, mice retreated with saline:PEG-20 Sorbitan Oleate (3:1) initially had no detectable antibody response (IgG and IgM) after injection of ovalbumin:saline or ovalbumin:$Al(OH)_3$. Mice reimmunized with the adsorbed antigen had significant increases in IgG and IgM antibody titers. Mice retreated with saline:PEG-20 Sorbitan Oleate and immunized with ovalbumin alone had no IgG_1 response throughout the study. After immunization with ovalbumin:$Al(OH)_3$ no IgG_1 response was detected until after reimmunization. The primary response (IgG and IgM) was not detected until reimmunization with any antigen after the mice were retreated with retinol:PEG-20 Sorbitan Oleate. The IgG_1 titer for mice treated with 1000 IU retinol:PEG-20 Sorbitan Oleate was initially less than that produced by saline:PEG-20 Sorbitan Oleate, but was the same by the end of the study. Pretreatment with 3000 to 9000 IU of retinol:PEG-20 Sorbitan Oleate caused suppression of the primary response, but reimmunization produced high antibody titers.

In general, mice treated with RP on day −1 and immunized with ovalbumin:saline on days 0 and 30 had "excellent" IgE responses, but only after the second injection of the antigen. Retinol apparently promoted priming of IgE-specific components, whereas PEG-20 Sorbitan Oleate inhibited the other components of the immune response. Pretreatment with saline alone resulted in the production of "substantial amounts of antibodies." The investigators concluded that when retinol:PEG-20 Sorbitan Oleate was the sole source of adjuvant, a dose-dependent increase in the secondary response occurred; suppression of the primary adjuvant-independent response was not total. Some suppression of the primary immunoresponse occurred when the mice were immunized with ovalbumin:$Al(OH)_3$ after pretreatment with retinol:PEG-20 Sorbitan Oleate. The retinol portion of the adjuvant combination induced a secondary response that equaled the secondary response of the antigen alone. The primary IgG_1 response was totally inhibited by PEG-20 Sorbitan Oleate, and the secondary response was dependent on retinol. Retinol presumably acted upon the B-cell component.

TABLE 5
Immunosuppressive effects of adjuvants (Barnett and Bryant 1980)

Adjuvant	Antigen	Results
Saline (control)	OA:$Al(OH)_3$	10^3–>10^7 throughout study
Saline (control)	OA:saline	Similar results
SP (control)	OA:saline	No detectable IgG or IgM response at first immunization
SP (control)	OA	No IgG_1 response
SP (control)	OA:$Al(OH)_3$	No antibody responses until after reimmunization; IgG and IgM titer = 10^2–10^5 at weeks 4–9; peak IgG_1 titer = 80 at week 9
RP (1000 IU)	OA	No IgG_1 response
RP (3000 IU)	OA	No IgG_1 response
RP (5000 IU)	OA	No IgG_1 response until after reimmunization; peak titer = 80
RP (9000 IU)	OA	No IgG_1 response
RP (1000 IU)	OA:saline	Peak IgG and IgM titer = 160 at weeks 2–3; no response at weeks 4–6; second peak = 50 at week 9
RP (3000 IU)	OA:saline	Peak IgG and IgM titer = 10^5 at weeks 5–6
RP (5000 IU)	OA:saline	Peak IgG and IgM titer = 10^5 at weeks 5–6
RP (9000 IU)	OA:saline	Peak IgG and IgM titer = 10^3 at week 8
RP (1000 IU)	OA:$Al(OH)_3$	"Lagged behind" other groups in terms of IgG and IgM titer; titer = 100 at week 4, 10^3–10^8 for remainder of study
RP (3000 IU)	OA:$Al(OH)_3$	IgG and IgM titer = ~170 at week 4; 10^8 at week 9; peak IgG_1 titer = 640 at week 8
RP (5000 IU)	OA:$Al(OH)_3$	IgG and IgM titer = ~140 at week 3; ~1.7×10^7 at week 9; IgG_1 titer = 80 at week 5; 320 at week 7
RP (9000 IU)	OA:$Al(OH)_3$	IgG and IgM titer = 10–100 at weeks 3–4; ~1.7×10^3 at week 5; 10^4 at week 8; no IgG_1 response until week 5; peak titer = ~200 at weeks 8–9

IU = international units; 0.3 μg.
RP = retinol:PEG-20 Sorbitan Oleate.
OA = ovalbumin.
SP = saline:PEG-20 Sorbitan Oleate.

The majority of the observed immunosuppressive effects could be attributed to the Polysorbate, but retinol could also have been toxic. The investigators concluded that either (a) T-helper cell function was affected or (b) the surfactant properties of PEG-20 Sorbitan Oleate prevented activation of macrophages and/or their interaction with T-helper and B cells (Barnett and Bryant 1980).

In a follow-up study by Barnett (1981), Balb/c mice treated with PEG-20 Sorbitan Oleate (emulsified in saline, 3:1) had significantly decreased numbers of primary (IgM) plaques without a significant change in the number of secondary (IgG) plaques during a Jerne plaque assay. For this assay, the mice (number not available) were immunized on day 0 with 2×10^7 sheep erythrocytes administered by the IP route. On day 5, half of the mice per group was killed and assayed for the number of IgM antibody-producing cells in their spleens using a direct hemolytic plaque assay. On day 10, the secondary immune response was boosted in the remaining mice with a second IP administration of sheep erythrocytes. On day 14, the mice were killed, and the secondary IgG response was quantified via the indirect hemolytic plaque assay (using rabbit anti-mouse IgG).

The effect of PEG-20 Sorbitan Oleate on cell-mediated immunity was investigated for this study using the delayed hypersensitivity response to a contact allergen. After treatment, as above, with PEG-20 Sorbitan Oleate, mice were sensitized on day 0 by topical application of 25 μl of 8% (w/v) oxazolone in acetone. One day 5, the mice were challenged with 10 μl of 0.1% oxazolone by topical application to the dorsal surface of the right external ear. Immunologic responses to the allergen were determined for three days using micrometer measurements of the increase in external ear thickness. PEG-20 Sorbitan Oleate had no significant effect on the contact hypersensitivity response; the Polysorbate had no effect on the priming and triggering of T-effector cells. The investigator concluded that the inhibition of the primary, and not the secondary, hemolytic plaque response involved inhibition of the primary IgE antibody response as well as a decrease of the concentration of circulating IgG and IgM antibodies.

PEG-20 Sorbitan Oleate was a histamine-releasing agent (Eschalier et al. 1988). In a study on the Polysorbate's effects on macrophage activation (Bonhomme et al. 1993), 1% PEG-20 Sorbitan Oleate increased peritoneal macrophage recruitment

without modifying phagocytic activity. This study used female Swiss mice that were injected IP with 2 ml volumes of the sterilized test chemicals. Four days after dosing, the peritoneal macrophages were harvested and viable cells were counted. Macrophage phagocytic activity was measured in terms of chemiluminescence following engulfment of opsonized zymosan.

Miscellaneous Effects

During a malabsorption study (Ohsumi et al. 1979), Wistar rats were fed 5% to 10% PEG-20 Sorbitan Oleate for 3 months. The body weight was determined weekly, and the effects of the detergents on the small intestine were observed at 1 week, 2 months, and 3 months after the treatment. In addition, glucose absorption was evaluated after the infusion of glucose, sucrose, and dextrin solutions into the rat intestine. The goblet cells, intestinal epithelial cell, and villi were evaluated using light microscopy, and the brush border and mitochondria were examined using electron microscopy. The villi were also examined using scanning electron microscopy. One week after treatment with PEG-20 Sorbitan Oleate, glucose absorption was greater than after treatment with the control vehicle. At 3 months, the blood glucose concentrations were lower in rats given the Polysorbate. Intestinal epithelial cells of rats treated with 5% PEG-20 Sorbitan Oleate were intact, whereas the mitochondria were "destructed" and the cristae were distributed irregularly. For 10% PEG-20 Sorbitan Oleate, a portion of the microvilli disappeared, and the surface of the epithelial cells appeared flat. Changes of the mitochondria occurred (giant mitochondria, destruction of cristae), and vacant sacks were observed in the cells.

Using a cascade superfusion bioassay system, Uluoglu et al. (1996) investigated the functional reduction of endothelial function in rabbit thoracic aorta by PEG-20 Sorbitan Oleate. The bioassay tissue was deendothelized precontracted aorta ring from male albino rats. Segments of rabbit thoracic aortas (from which the fat and connective tissue were removed) were obtained from male New Zealand white rabbits. The segments were either incubated with 1% to 10% PEG-20 Sorbitan Oleate for 30 minutes or 3 hours (or Kreb's solution as a control) before being placed in a donor chamber, or were placed in the chamber without previous incubation. Half of the segments that was not incubated were perfused with Kreb's solution containing 10^{-1} to 10^{-3} ml/l PEG-20 Sorbitan Oleate (perfusion rate = 3 ml/min); the remaining half served as a control and was perfused with Krebs solution alone. The bioassay rings were superfused with the effluents of the rabbit aortas, and removal of endothelium was determined by brief exposure of the bioassay rings to acetylcholine. Incubation with 10% PEG-20 Sorbitan Oleate and perfusion with 10^{-1} ml/l PEG-20 Sorbitan Oleate caused total inhibition of the release of acetylcholine-induced endothelium derived relaxing factor (EDRF). At microscopic examination, significant desquamation of vascular endothelium was observed after treatment with PEG-20 Sorbitan Oleate, but no damage to the underlying smooth muscle was observed. Low to moderate concentrations of the Polysorbate had no significant effect on the release of EDRF. Perfusion with PEG-20 Sorbitan Oleate also inhibited EDRF release from donor aorta in a dose-dependent manner. The investigators suggested that the endothelial lining could have been denuded by PEG-20 Sorbitan Oleate, resulting in the reduction in the release of EDRF.

PEG-20 Sorbitan Oleate and PEG-20 Sorbitan Stearate released lysosomal enzymes from the intestinal mucosal cells of the female Sprague-Dawley rat. The intestinal permeability was slightly increased to sodium fluorescein in the absence and presence of the Polysorbates at concentrations of 10 mg/ml (instilled into a section of ligated, cannulated gut). The investigators concluded that surface-active agents had the potential to impair the function of the mucosal barrier and to increase the permeability of the gut to potentially toxic and pathogenic compounds (Tagesson and Edling 1984).

Oberle, Moore, and Krummel (1995) reported that surfactants, including PEG-20 Sorbitan Oleate, increased the rates of lactate dehydrogenase and mucus release in the jejunum and colon of male Sprague-Dawley rats (four to nine per group). The lactate dehydrogenase release rate in the jejunum increased approximately twofold after perfusion of 1% PEG-20 Sorbitan Oleate, compared to saline controls. Minimal morphological damage was observed using light and scanning electron microscopy. The enzyme release rate was approximately threefold less in the colon than jejunum in saline and 1% PEG-20 Sorbitan Oleate, but the rate was twofold greater in rats treated with the Polysorbate than in rats of the control group. Total tissue lactose dehydrogenase decreased along the length of the intestine and was approximately sevenfold less in the colon than in the jejunum, indicating that the relative effects of the surfactants were similar between intestinal regions. Release rates were linear for 6 hours with both saline and PEG-20 Sorbitan Oleate. Mucus release in the jejunum was greater after exposure to PEG-20 Sorbitan Oleate.

In a study by Chi, Scocca, and Huang (1978), PEG-20 Sorbitan Oleate did not increase or diminish the effects of UV and gamma irradiation in *Escherichia coli.*

PEG-20 Sorbitan Oleate caused the proliferation and aggregation of *E. coli* K-12. Normally, *E. coli* grows in culture in a dispersed state (Levinson, Allen, and Sung 1978). In this study, maximal aggregation and proliferation occurred when a concentration of 0.005% PEG-20 Sorbitan Oleate was added to the minimal medium.

PEG-20 Sorbitan Oleate had an immediate effect on the permeability barrier of three strains of *Pseudomonas aeruginosa*, but did not affect that of *E. coli* (Brown and Winsley 1969). In this study, effects on cell permeability were assessed by measuring the effects on the leakage of 260 nm–absorbing substances (on entry of a fluorescent dye) and by the viability of bacteria "under stress." Leakage was enhanced when the cells were treated with concentrations up to 1.25% of PEG-20 Sorbitan Oleate. Cells of *P. aeruginosa* treated with the Polysorbate leaked more readily and had greater percentage viability losses when the pH, temperature, or sodium chloride concentration (suspending fluid) was changed rapidly.

PEG-20 Sorbitan Oleate and PEG-20 Sorbitan Laurate caused 30% to 100% inhibition of the spasmogenic effects of histamine, acetylcholine, carbachol, angiotensin, and other compounds in isolated guinea pig ileum and isolated rabbit jejunum (Sabir, Singh, and Bhide 1972).

Gough et al. (1982) reported that PEG-20 Sorbitan Oleate was a potent cardiac depressant in dogs. The Polysorbate was dissolved in deionized water to give a concentration of 100 mg/ml, and was injected intravenously over a 5-minute period. In addition, commercial IV amiodarone (containing 50 mg/ml amiodarone and 100 mg/ml PEG-20 Sorbitan Oleate) was similarly injected. Within 5 minutes of injection of PEG-20 Sorbitan Oleate, blood pressure, left ventricular maximum dP/dt, and cardiac output were significantly decreased in all four treated dogs. Within 5 minutes of injection of the commercial solution, the three dogs tested had "severely reduced" mean arterial blood pressure and left ventricular maximum dP/dt. These parameters remained reduced for >1 hour. Cardiac output also decreased, but due to the variation in control values, the significance could not be determined. For both test compounds, >75% reduction remained in left ventricular dP/dt after 30 minutes; blood pressure was reduced by at least 68%.

PEG-20 Sorbitan Oleate also caused hemodynamic changes in approximately 20% of 33 guinea pigs treated with 6 ml of 0.02% PEG-20 Sorbitan Oleate in isotonic saline via injection into the left atrium. Within 30 seconds of injection, coronary flow was reduced to an average of 30% below control. This reduction lasted for 0.5 to 3 minutes, and was followed by a subsequent hyperemic response in which peak flow averaged 53% above control. Normal flow resumed by 9 minutes after injection. Left ventricular pressure, aortic blood pressure, and left ventricular stroke volume only declined slightly, and heart rate was unaffected. Upon a second injection, only seven guinea pigs had recurrent adverse reactions (Grund et al. 1995).

The Polysorbates have been reported to activate or inhibit various in vitro biochemical reactions. Enzyme activities affected by these ingredients included those of biophenyl 4-hydroxylase, glucose-6-phosphate dehydrogenase, cholesterol oxidase, Na/K ATPace Mg^{2+}, ATPase, dimethylnitrosamine demethylase, ethylmorphine demethylase, dichloro-*p*-nitro-anisole *O*-demethylase, aniline 4-hydroxylase, biphenyl 2- and 4-hydroxylase, palmityl coenzyme A carnitine *O*-palmityltranferase, and acetylcholinesterase, as well as other enzymes. The Polysorbates affected cellular respiration in rat small intestine epithelial cells by inhibiting oxygen consumption. Lactic acid formation was increased by low concentrations and decreased by high concentrations of PEG-20 Sorbitan Laurate or PEG-20 Sorbitan Oleate. The latter ingredient slightly inhibited mitochondrial oxidation by progressively decreasing phosphorylation capacity with increasing concentration of the Polysorbate. PEG-20 Sorbitan Laurate inhibited the spasmogenic effects of acetylcholine, barium chloride, and histamine during in vitro studies using isolated guinea pig duodenum and ileum. PEG-20 Sorbitan Laurate also stimulated secretion of bile when injected intraduodenally (1 ml/kg) into rats. The Polysorbates are nonspecific histamine releasers. Intravenous infusion of PEG-20 Sorbitan Laurate (5 ml, 0.2 ml/15 s) into splenectomized dogs produced anaphylactic-like clinical signs that could have been mediated by endogenous histamine release. The Polysorbates affected the structure and function of cellular membranes. PEG-20 Sorbitan Laurate caused the lysis of erythrocytes and, in a study using artificial membranes, penetrated a lecithin monolayer to block charge transfer through the interface. It also increased membrane resistance and decreased membrane stability of a bimolecular oxidized cholesterol membrane. Investigators suggested that Polysorbates lower conductance of the membrane by making it less permeable to charged molecules and decrease membrane stability when incorporated into the membrane structure (Elder 1984).

Skin penetration of lidocaine increased generally in the presence of an aqueous propylene glycol vehicle containing 1% PEG-20 Sorbitan Laurate or PEG-20 Sorbitan Stearate (Sarpotdar and Zatz 1986). The addition of either Polysorbate to a 40% aqueous solution of propylene glycol caused a decrease in lidocaine penetration rate, however. In solutions containing 60% propylene glycol, flux was greater in the solution containing Polysorbate than the control solution with no surfactant. An 80% solution of propylene glycol with the surfactant had three times the flux of the control solution. In the 40% solution, the micellar solubilization of lidocaine lowered its activity in the vehicle, hence the decrease in its penetration rate.

PEG-20 Sorbitan Oleate (9%; 5 ml/kg IV) affected the blood-brain barrier in an in situ brain perfusion assay such that the Polysorbate enhanced the brain uptake and analgesic activity of D-kyotorphin in mice and rats (Sakane et al. 1989).

The Polysorbates influenced the transport of larger molecules across membranes and thus affected drug activity and toxicity (Elder 1984). When Polysorbate-type surfactants were used as emulsifiers and stabilizers in foodstuffs and food colorants, toxic synergy occurred in golden hamsters. PEG-20 Sorbitan Oleate, however, decreased the acute oral toxicity in mice of tetracycline, norsulfazole, theophylline, tubazid, procaineamide, amidopyrine, and pentobarbitol. In other studies, PEG-20 Sorbitan Oleate decreased percutaneous penetration of butylparaben in vitro through guinea pig skin and inhibited absorption of *p*-aminobenzoic acid from the in situ small intestine of the rat, despite the increase in solubilized concentration and membrane permeability of the compounds, respectively, caused by PEG-20 Sorbitan Oleate.

Sorbitan Laurate at concentrations of 1% to 60% in petrolatum or water was applied daily to the clipped backs of New Zealand white rabbits for 81 days. Treated skin sites had increased numbers of inflammatory cells in the dermis. Oxygen consumption of skin treated with Sorbitan Laurate for 3 to 13 days increased twofold; at 30 to 81 days of treatment the increase was two- to threefold (Mezei et al. 1966).

ANIMAL TOXICOLOGY

The available studies on the PEGs Sorbitan/Sorbitol Fatty Acid Esters (including the Polysorbates), PEGs, and Sorbitan Esters are summarized in Table 6.

TABLE 6
Toxicology data summary

Test chemical	In vitro	Animal	Clinical
		New or updated data	
PEG-20 Sorbitan Laurate	Cytotoxicity	Behavioral effects	Parenteral toxicity
	Primary skin irritation	Short-term toxicity (IV, IP)	
	Ocular irritation	Primary skin irritation	
	Genotoxicity	Ocular irritation	
		Comedogenicity	
		Reproductive and developmental toxicity	
		Tumor inhibition	
PEG-20 Sorbitan Stearate	Cytotoxicity	Ocular irritation	Sensitization
	Primary skin irritation	Reproductive and developmental toxicity	
	Ocular irritation	Cocarcinogenicity	
	Metabolic cooperation	Carcinogenicity	
	Genotoxicity	Tumor inhibition	
PEG-20 Sorbitan Oleate	Mucosa permeability effects	Immunosuppressive effects	Parental toxicity
		GI and cardiac effects	Sensitization
	Cytotoxicity	Penetration enhancement	
	Primary skin irritation	Acute toxicity (ICV,* IP)	
	Ocular irritation	Behavioral effects	
	Genotoxicity	Short-term toxicity (IV, IP, oral)	
	Metabolic cooperation	Subchronic toxicity (oral)	
		Skin sensitization	
		Ocular irritation	
		Pulmonary toxicity	
		Reproductive and developmental toxicity	
		Carcinogenicity	
		Tumor inhibition	
PEG-20 Sorbitan Palmitate	Cytotoxicity	Ocular irritation	Sensitization
	Primary skin irritation	Tumor inhibition	
	Ocular irritation		
	Genotoxicity		
PEG-80 Sorbitan Palmitate		Primary skin irritation	
		Ocular irritation	
PEG-40 Sorbitan Lanolate		Acute toxicity (IP)	Sensitization
		Primary skin irritation	
		Ocular irritation	
PEG-50 Sorbitol Hexaoleate		Acute toxicity (IP)	Sensitization
		Primary skin irritation	
		Ocular irritation	
PEG-30 Sorbitol Tetraoleate Laurate		Acute toxicity (IP)	Sensitization
		Primary skin irritation	
		Ocular irritation	
PEG-40 Sorbitan Peroleate	Genotoxicity	Acute toxicity (IP)	Sensitization
		Ocular irritation	
PEG-20 Sorbitan Trioleate	Primary skin irritation	Acute toxicity (intramuscular)	
	Ocular irritation		
Sorbitan Esters		Acute toxicity (intramuscular, oral)	Sensitization
		Primary skin irritation	Primary and cumulative irritation

TABLE 6
Toxicology data summary *(Continued)*

Test chemical	In vitro	Animal	Clinical
		Data from previous safety assessments	
PEG-4 Sorbitan Laurate		Acute toxicity (oral, IP, IV) Chronic toxicity (oral) Primary skin irritation Ocular irritation Tumor inhibition	
PEG-20 Sorbitan Laurate	Membrane permeability Effects on biochemical reactions	Metabolism, distribution, excretion Acute toxicity (oral, IP, percutaneous, IV Short-term toxicity (oral) Subchronic toxicity (oral) Chronic toxicity (oral) Primary skin irritation Sensitization Ocular irritation Carcinogenicity	Primary skin irritation Sensitization Photosensitization Ocular irritation
PEG-4 Sorbitan Stearate		Acute toxicity (oral) Short-term toxicity (oral) Subchronic toxicity (oral) Chronic toxicity (oral) Ocular irritation	
PEG-20 Sorbitan Stearate	Effects on biochemical reactions	Absorption, metabolism Acute toxicity (oral) Short-term toxicity (oral) Chronic toxicity (oral) Primary skin irritation Ocular irritation Carcinogenicity Cocarcinogenicity Tumor inhibition	Oral toxicity Primary skin irritation Cumulative skin irritation Sensitization
PEG-5 Sorbitan Oleate		Acute toxicity (oral) Short-term toxicity (oral) Primary skin irritation Ocular irritation Carcinogenicity	
PEG-20 Sorbitan Oleate	Effects on biochemical reactions Genotoxicity	Absorption, metabolism, excretion Penetration enhancement Acute toxicity (oral) Short-term toxicity (oral) Subchronic toxicity (percutaneous) Chronic toxicity (oral) Sensitization Reproductive and developmental toxicity Carcinogenicity Cocarcinogenicity Tumor inhibition	Oral toxicity Primary skin irritation Sensitization
PEG-20 Sorbitan Palmitate	Effects on biochemical reactions	Acute toxicity (oral) Short-term toxicity (percutaneous) Chronic toxicity (oral) Primary skin irritation	Cumulative skin irritation

(Continued on next page)

TABLE 6
Toxicology data summary *(Continued)*

Test chemical	In vitro	Animal	Clinical
		Ocular irritation	
		Carcinogenicity	
		Cocarcinogenicity	
		Tumor inhibition	
PEG-20 Sorbitan Trioleate		Acute toxicity (oral)	Primary skin irritation
		Chronic toxicity (oral)	Cumulative skin irritation
		Ocular irritation	Sensitization
		Carcinogenicity	
PEG-20 Sorbitan Tristearate		Absorption, metabolism	
		Acute toxicity (oral)	
		Chronic toxicity (oral)	
		Sensitization	
		Ocular irritation	
		Carcinogenicity	
Sorbitan Esters	Genotoxicity	Absorption, metabolism, distribution	Oral toxicity
		Effects on skin structure and O_2 consumption	Primary skin irritation
		Acute toxicity (oral, intramuscular)	Cumulative skin irritation
		Short-term toxicity (oral)	Sensitization
		Subchronic toxicity (oral)	Phototoxicity
		Chronic toxicity (oral)	Photosensitization
		Ocular irritation	
		Carcinogenicity	
		Primary skin irritation	
PEGs	Genotoxicity	Absorption, metabolism, excretion	Primary skin irritation
		Acute toxicity (oral, dermal)	Sensitization
		Short-term toxicity (dermal)	
		Subchronic toxicity (oral)	
		Chronic toxicity (oral)	
		Primary skin irritation	
		Sensitization	
		Ocular irritation	
		Reproductive and developmental toxicity	
		Carcinogenicity	

*ICV = intracerebroventricular.

Acute Toxicity

PEGs Sorbitan/Sorbitol Fatty Acid Esters

Fifteen female rats were given 39.8 g/kg PEG-40 Sorbitan Lanolate and five were given 25.1 g/kg of the compound via gastric intubation. The investigators observed signs of intoxication including depression, diarrhea, and stained, wet perineal areas, but none of the rats died before their scheduled necropsy. Gross lesions included hydronephrosis, focal congestion of the lungs and thymus, and congestion of the mesenteric lymph nodes. PEG-40 Sorbitan Lanolate was classified as "relatively harmless" (CTFA 1998b).

When PEG-50 Sorbitol Hexaoleate and PEG-30 Sorbitol Tetraoleate Laurate were tested similarly using rats and mice, the LD_{50} values were >31.6 g/kg. The LD_{50} of PEG-40 Sorbitan Peroleate in five male and five female rats was greater than 28.2 g/kg (CTFA 1998b).

Polysorbates

The acute oral LD_{50} values of PEG-20 Sorbitan Laurate were >38.9 g/kg in rats and >25 g/kg in mice. In dermal studies, adverse effects were not observed after undiluted PEG-20 Sorbitan Laurate was injected percutaneously into the intact or abraded skin of albino guinea pigs. No signs of toxicity were observed after guinea pigs were immersed for 4 h/day in a 0.5% aqueous solution of a product formulation containing 8.4% PEG-20 Sorbitan Laurate for 3 consecutive days. The parenteral LD_{50} of PEG-20 Sorbitan Laurate in rats was 0.7 ml/kg or 1.45 g/kg (IV). In mice, these values ranged from 1.42 to 3.75 g/kg. When

administered IP, the LD_{50} in rats was 3.85 g/kg; in mice, the LD_{50} was 2.64 g/kg. The acute oral LD_{50} of PEG-4 Sorbitan Laurate in the rat was >38 g/kg. The LD_{50} values of PEG-4 Sorbitan Laurate in the rat were 1.38 g/kg (IV) and >5 ml/kg (IP). The acute oral LD_{50} values of PEG-20 Sorbitan Palmitate and PEG-4 and -20 Sorbitan Stearate in the rat varied from >5 g/kg to >38.4 g/kg. For PEG-20 Sorbitan Tristearate, the LD_{50} was >10 g/kg to >39.8 g/kg. In rats the LD_{50}s for PEG-5 Sorbitan Oleate ranged from 20 ml/kg to >36.6 g/kg, and the LD_{50} for PEG-20 Sorbitan Oleate ranged from 20 ml/kg to 54.5 ml/kg. In mice, the LD_{50} for PEG-20 Sorbitan Oleate was >25 g/kg (Elder 1984).

The acute IV LD_{50} of PEG-20 Sorbitan Oleate was 1790 mg/kg in the rat. The IP LD_{50} values were 8210 mg/kg in the mouse and 6804 mg/kg in the rat. The acute oral LD_{50} in the mouse was 25 g/kg. The IV LD_{LO} values of PEG-20 Sorbitan Oleate in the mouse, dog, and cat were 1 g/kg, 500 mg/kg, and 500 mg/kg, respectively (Radian Corporation 1991).

Dib and Falchi (1996) gave Ofa albino rats intracerebroventricular injections of 5% PEG-20 Sorbitan Oleate in saline, with or without an ethanol emulsion. Thirty-six rats of group 1 were injected with 10 μl PEG-20 Sorbitan Oleate and 5% ethanol. Within 2 to 3 minutes after injection, the rats had strong convulsions and died; blood mixed with mucus was observed around the nasal area. Seven rats of group 2 were treated with the Polysorbate and 0.5% ethanol. Two rats had convulsions and died within 5 minutes of treatment, and the others recovered locomotory activity within an hour. Two rats of group 3 had an injection of the Polysorbate without ethanol. The rats "lay quietly" in their cages during the initial five minute period, then died after convulsions. The six rats injected with 10 to 20 μl of 5% ethanol alone were not affected. When capsaicin was dissolved in PEG-20 Sorbitan Oleate, no adverse effects were noted.

The behavioral effects of PEG-20 Sorbitan Laurate and PEG-20 Sorbitan Oleate were evaluated using 8 to 12 male CD2F1 mice per group (Castro et al. 1995). The Polysorbates were administered at various concentrations by IP injection; the dose volume was 10 ml/kg. The mice were observed for 12 hours after treatment. PEG-20 Sorbitan Laurate significantly decreased locomotor activity (by 50% of controls) at a concentration of 16% in saline (*v/v*). PEG-20 Sorbitan Oleate decreased locomotor activity at a concentration of 32% in saline (*v/v*).

A volume of 0.5 ml PEG-20 Sorbitan Trioleate was injected $^1/_2$ inch deep in the right and left pectoral muscles of six 7 to 8-week-old male Hubbard Crossbred chickens (Hem et al. 1974, 1975). One chicken had inflammation of tissue involving >8.1 cm^2, one had inflammation involving <2.0 cm^2, one had necrosis, and three had inflammation involving 2.1 to 8.0 cm^2. The same results were observed at the left and right injection sites for each chicken.

Sorbitan 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 non-toxic" under the conditions of the study (CTFA 1998c).

No toxic effects were observed in 10 male rats that were given 20 g/kg Sorbitan Laurate as a single oral dose. The LD_{50} values of Sorbitan Laurate determined by acute oral toxicity studies using rats ranged from 33.6 to 41.5 g/kg. Sorbitan fatty acid esters in low concentrations were relatively nontoxic after ingestion. The lowest reported rat LD_{50} in twenty studies was 31 g/kg for Sorbitan Stearate (Elder 1985).

Male Hubbard Crossbred chickens (6/group) which had Sorbitan Trioleate injected $^1/_2$ inch deep into the right and left pectoral muscles had inflammation and necrosis of the tissue at the injection site within seven days of treatment (Hem et al. 1974, 1975). One chicken had inflammation involving <2.0 cm^2 at both sites, one chicken had inflammation involving <2.0 cm^2 at the left site and inflammation involving 2.1 to 8.0 cm^2 at the right injection site. One chicken had inflammation involving 2.1 to 8.0 cm^2 at both sites, one had >8.1 cm^2 inflammation at the left site and necrosis at the right site, and one chicken had necrosis at both sites. Four chickens injected with Sorbitan Isostearate had inflammation of the injection site: three had 2.1 to 8.0 cm^2 inflammation at both sites and one had >8.1 cm^2 at both sites. One chicken had no visible signs of tissue damage at either site, and one had 2.1 to 8.0 cm^2 inflammation at one site, but no signs of tissue damage at the other.

Polyethylene Glycol

The acute oral LD_{50} in rabbits of 100% PEG-6 was 17.3 g/kg; that of 100% PEG-75 was 76 g/kg. Acute dermal toxicity studies did not result in mortality after rabbits were given 20 ml/kg doses of undiluted PEG-6 or 40% PEG-20M (Andersen 1993).

Short-Term Toxicity

Polysorbates

A parenteral vitamin E supplement containing Polysorbate emulsifiers was implicated in a number of deaths in premature infants (see Clinical Assessment of Safety—Parenteral Toxicity). To study the toxicity of this supplement, newborn (1-week-old) albino rabbits were given IV doses of 4 ml/kg/day (100 mg/kg/day) of one of two vitamin E preparations containing 9% PEG-20 Sorbitan Oleate and 1% PEG-20 Sorbitan Laurate in water, or the Polysorbate vehicle alone. Parenteral nutrition was provided constantly. To determine the influence of diet, rabbits in both the treatment and control groups received either a low-energy (LE) diet or high-energy (HE) diet. The LE diet was the standard pediatric IV nutrient solution given to premature infants. The HE diet was similar in composition to rabbit milk. Rabbits given the LE diet gained weight at an average of 0.6 ± 1.0 g/day, and rabbits given the HE diet gained an average of 3.9 ± 0.5 g/day. After 6 to 7 days of treatment, the rabbits were killed and the blood and tissues were analyzed. Rabbits of all treatment and control groups given the LE diet had centrilobular degeneration and necrosis and pigment accumulation in the liver. No other lesions were attributed to the administration of the vitamin E preparations or the Polysorbate vehicle.

Rabbits given the HE diet had no nutrition-related centrilobular degeneration, but they had other treatment-related changes. These changes included microscopic evidence of mild bile stasis, elevated serum bilirubin, and minimal lipidosis of the liver, spleen, and adrenal cortex. The study suggested that vitamin E (α-tocopherol or α-tocopherol acetate) in a Polysorbate vehicle was mildly hepatotoxic and altered fat metabolism (Rivera et al. 1990).

Farkas et al. (1991) treated neonatal Sprague-Dawley rats and 566 mice with the same Polysorbate vehicle by IP injection. The strain of mouse and number of rats were not available. The vehicle was diluted to 10% in saline. In an acute toxicity study using rats, essentially all mortality was in the first 7 days. Multiple injections during a 90-day study had little effect on increasing mortality if the treated rats lived 7 days; the injections, however, produced massive peritoneal fibrosis and adhesions between organs in rats that lived for 2 to 3 weeks. Rats treated with 3.5 to 4 g/kg had swollen, inflamed tails within several days of the injection, and annular constrictions developed. In newborn mice injected daily, gross or microscopic evidence of hepatic damage was not observed. The mortality pattern paralleled that observed in rats. Animals injected with $\geq$2.5 g/kg had chylous ascites and hydropic degeneration of the renal tubules. Renal tubular regeneration was observed in rats and mice that survived multiple injections of the Polysorbates. Large areas of Zenker's necrosis of the muscle of the diaphragm were observed at microscopic examination at the same time of the appearance of chylous ascites.

A body lotion containing 4% PEG-20 Sorbitan Palmitate was tested for percutaneous toxicity during a 28-day study. Doses of 0.3 or 0.9 ml/kg/cm^2 were applied daily (5 days/week) to the backs of albino rabbits, half of which received epidermal abrasions twice a week. After 2 weeks of treatment, several rabbits had slight peripheral leukocytosis, and dose-related dermatitis (mild to moderate erythema and edema and scaly desquamation) was observed. No adverse effects were reported when chicks were given 0.1 to 2% PEG-20 Sorbitan Laurate in feed for 7 weeks. In feeding studies using rats, 3% to 5% PEG-20 Sorbitan Laurate caused slow weight gain attributed to mild diarrhea, but no other signs were noted after eight weeks of treatment. In a 10-week study using hamsters, 5% to 15% PEG-20 Sorbitan Laurate caused high mortality that could have been due to diarrhea. No adverse effects were noted when PEG-20 Sorbitan Stearate was fed to chicks at up to 2% for 7 weeks or to rats fed up to 5% for 8 weeks. Rats fed 10% PEG-20 Sorbitan Stearate for 8 weeks had diarrhea after the first few days, with recovery after continued feeding. During a 3-month study, Charles Foster rats fed 1.5 ml PEG-20 Sorbitan Oleate at 1% to 4% had congestion and degenerative changes in the heart, liver, and kidneys, which were attributed to capillary wall damage. In 6-week studies the by the same investigators, rats given 1% to 4% PEG-20 Sorbitan Stearate and PEG-5 Sorbitan Oleate and rhesus monkeys given 2 ml/day PEG-4 Sorbitan Stearate had no adverse effects. In these studies, the observed diarrhea was considered likely due to the high concentrations of the unabsorbed polyoxyethylene sorbitan moiety within the intestinal lumen. It could have been directly or indirectly the cause of the other adverse effects (retarded growth, etc.) observed in the feeding studies (Elder 1984).

During a 14-day feeding study performed by the National Toxicology Program (NTP 1992b), five rats and five mice per sex per group were fed 3000, 6000, 12,500, 25,000, or 50,000 ppm/day PEG-20 Sorbitan Oleate. No deaths occurred prior to study termination. The mean body weight change of males fed 50,000 ppm was significantly lower than that of controls. No clinical findings related to administration of the test compound were observed.

Sorbitan Esters

The feeding of Sorbitan Stearate to rats for 8 weeks did not affect growth; other studies indicated that the ingredient had nutritive value for rats and dogs. A slight but inconsistent (nonsignificant) increase in growth occurred in chicks fed 0.1% to 2.0% Sorbitan Laurate for 10 weeks, with or without penicillin supplementation. Mortality, body weight, and necropsy findings were not affected by treatment. Rats (three groups of 12) fed 1% to 4% Sorbitan Laurate for 6 weeks had slightly decreased growth rates; all other parameters were normal. Rhesus monkeys given 2 g/day Sorbitan Laurate for 6 weeks had no signs of toxicity. Hamsters fed 5% Sorbitan Laurate for 68 days had reduced growth rates and slightly greater mortality than controls. Hamsters fed 15% had diarrhea, higher mortality, and gastrointestinal mucosal hyperemia, edema, and renal tubule epithelial degeneration. Increased weights of the brain, kidneys, heart, spleen, lungs, and liver were observed in Sprague-Dawley rats fed 25% Sorbitan Laurate for 59 days. Prior to necropsy, the rats had reduced body weights, diarrhea, nasal hemorrhage, and gangrenous tails. In a 70-day feeding study, the rats had decreased activity and appetite, reduced weight gain, nasal bleeding, gangrene of the tail and hind legs, increased organ weights, and degenerative changes of the gastrointestinal tract, kidneys, and liver (Elder 1985).

Polyethylene Glycol

No evidence of toxicity was observed in rabbits that had received daily dermal applications of PEG-20M (0.8 g/kg/day) for 30 days; however, transient, mild erythema was observed. The only evidence of systemic toxicity that resulted from dermal exposure was in rabbits that received repeated applications of an antimicrobial cream containing 63% PEG-6, 5% PEG-20, and 32% PEG-75 to *excised skin* for 7 days (Andersen 1993).

Subchronic Toxicity

Polysorbates

When 25% PEG-20 Sorbitan Laurate was fed to 10 rats over 21 weeks, there was only 1 fatality; however, significant gross and microscopic changes were observed in the urinary bladder, spleen, kidneys, and gastrointestinal tract. No adverse effects were reported when mice were fed 2.5% to 10% PEG-20

Sorbitan Stearate for 12 to 16 weeks. Mice given the compound at a concentration of 20% had some gastrointestinal "disturbance" with reduced feed intake and growth retardation. When rats were fed 5% to 10% (in soybean meal) PEG-20 Sorbitan Stearate for 14 weeks, no adverse effects were observed. When administered in purified casein, 5% caused diarrhea and growth retardation. Monkeys fed 2 g/day for 14 weeks had no adverse effects, and rats given 25% had growth retardation and transient diarrhea after 15 weeks of treatment. As noted above (see Short-Term Toxicity), the observed diarrhea was considered likely due to the high concentrations of the unabsorbed PEG sorbitan moiety within the intestinal lumen. A cream formulation containing 2.5% PEG-20 Sorbitan Oleate was tested for percutaneous toxicity. Doses of 6 mg/cm^2 were applied to the backs of rabbits for 90 consecutive days. No signs of systemic toxicity were observed, but moderate edema and erythema, slight to moderate desquamation, and mild dermatitis were noted by investigators. Slight erythema and scaly desquamation were reported when a cream formulation was tested at doses of 0.36 ml/260 cm^2/3 kg rabbit for 93 consecutive days (Elder 1984).

When diets containing 3100 to 50,000 ppm PEG-20 Sorbitan Oleate were fed to groups of 10 F344/N rats and 10 B6C3F$_1$ mice of each sex, all animals survived to study termination. No clinical findings, changes in absolute or relative organ weights, changes in mean body weight, or gross or microscopic lesions were observed during the 13-week study (NTP 1992b).

Sorbitan Esters

In subchronic studies, no toxic effects were noted when chickens, rats, monkeys, and hamsters were fed Sorbitan Laurate at concentrations < 10%. At greater concentrations, growth depression, decreased organ weights, diarrhea, unkempt appearance, hepatic and renal abnormalities, and gastrointestinal tract irritation were generally observed. Rats fed <10% Sorbitan Oleate had no abnormalities. At greater concentrations, the same types of abnormalities were observed as were noted in animals fed Sorbitan Laurate. No deaths occurred in Wistar rats fed 2.5% to 10.0% Sorbitan Laurate for 90 days. Treated rats had decreased body weights, hemoglobin concentrations, and packed cell volume values. The average weights of the brain, liver, and kidneys increased, but the average weights of the heart and gastrointestinal tract decreased. Periportal vacuolization of hepatocytes and tubular nephrosis were also observed. In a 13-week feeding study using rats, increased liver and kidney weights, and decreased body weights were observed following treatment with 10% Sorbitan Laurate. In a 23-week study, rats fed 15% to 25% Sorbitan Laurate had diarrhea, unkempt appearance, and severely retarded growth. Eight of ten rats of the high dose group died prematurely. A pale and enlarged liver, enlarged common bile duct, and gangrene of the tail were observed. Hepatic lesions included fatty changes, fibrosis, chronic inflammation, and necrosis. Other lesions observed were focal nephritis, increased numbers of foamy alveolar macrophages, and hyperplasia of cells of the bone marrow and spleen. Rats fed 10% Sorbitan Laurate for 17 weeks had decreased body weights, packed cell volume, and hemoglobin values. Kidney and liver weights were significantly increased (Elder 1985).

Polyethylene Glycol

In 90-day oral toxicity studies involving groups of albino rats, the largest and smallest molecular weight PEGs tested (PEG-20M and PEG-6, respectively) did not induce toxicity or death when administered daily in the diet (PEG-20M) or in drinking water (PEG-6) at concentrations of 4% or less (Andersen 1993).

Chronic Toxicity

Polysorbates

Of hamsters fed 5% to 15% PEG-20 Sorbitan Laurate for 28 to 39 weeks, 18/30 (10 per group) died and significant gross and microscopic lesions were observed in the urinary bladder, kidneys, spleen, and gastrointestinal tract. In a 17-month study using monkeys, dietary administration of 1 g/day PEG-20 Sorbitan Laurate produced no signs of toxicity. Rats fed 0.5% to 2% PEG-20 Sorbitan Laurate over the course of a lifetime had no adverse effects. Rats fed 2% PEG-4 Sorbitan Laurate for 2 years had neither gross nor microscopic changes. Numerous chronic feeding studies were performed on PEG-4 and -20 Sorbitan Stearate. No adverse effects were reported after PEG-20 Sorbitan Stearate was administered for up to 2 years at concentrations up to 10% in the mouse, 5% in the rat, 10% in the dog, and 1% in the hamster. When fed at greater concentrations, the only effects observed were diarrhea and some growth retardation; in multigeneration studies, 20% PEG-20 Sorbitan Stearate caused minor effects on growth, longevity, and reproduction. When dogs were fed PEG-20 Sorbitan Tristearate for 12 months at concentrations of 13.5% and 34%, the high dose caused phosphate kidney stones as a result of dehydration, and both doses caused periods of dehydration and diarrhea. In 2-year studies using rats (four generation studies), concentrations of 10% to 20% caused diarrhea, 20% caused minor effects on growth, longevity, and reproduction, and 2% to 5% caused no adverse effects. No gross or microscopic anomalies were observed after rats were fed 2% PEG-20 Sorbitan Palmitate for 2 years. No adverse effects were noted when two monkeys were treated with 1 g/day PEG-20 Sorbitan Oleate for 17 months. Rats fed 2% to 5% for 2 years had no adverse effects. Doses of 10% and 20% caused diarrhea, and 20% resulted in some minor effects on growth, longevity, and reproduction during a multigeneration study. Rats fed 2% PEG-5 Sorbitan Oleate or PEG-20 Sorbitan Trioleate for 2 years also had no adverse effects (Elder 1984).

Sorbitan Esters

Chronic feed studies have been conducted with Sorbitan Stearate, Sorbitan Laurate, and Sorbitan Oleate. At 5%, Sorbitan Laurate and Sorbitan Oleate had no adverse effects on rats over a 2-year period. Dogs fed 5% Sorbitan Stearate for 20 months had no changes related to the test compound; ≥ 10% was required to produce depressed growth and hepatic and renal abnormalities. Mice were more sensitive to Sorbitan Stearate than rats. Growth

depression was observed in rats fed 0.5% Sorbitan Stearate, and 4% caused renal abnormalities as well (Elder 1985).

Polyethylene Glycol

Toxic effects were not observed in dogs that received 2% PEG-8, PEG-32, or PEG-75 in the diet for 1 year (Andersen 1993).

Skin Irritation and Sensitization

PEGs Sorbitan/Sorbitol Fatty Acid Esters

PEG-40 Sorbitan Lanolate, PEG-50 Sorbitol Hexaoleate, and PEG-80 Sorbitan Palmitate did not produce erythema or edema when applied undiluted to the intact and abraded skin of New Zealand white rabbits for 24 to 72 hours. The primary irritation index (PII) was 0. Undiluted PEG-30 Sorbitol Tetraoleate Laurate caused very slight to well-defined erythema within 24 hours of treatment; very slight erythema was still evident 72 hours after treatment. The PII was 0.83/8, and the compound was classified as slightly irritating to the skin of rabbits (CTFA 1998b).

Polysorbates

In primary dermal irritation studies (by Draize methods) using rabbits, minimal to no irritation was observed after administration of undiluted PEGs-4 and -20 Sorbitan Laurate for up to 72 hours. In a 30-day dermal irritation study, PEG-20 Sorbitan Laurate caused erythema by the third day and skin thickening by day 10. At that time, minimal to mild inflammation was observed, but not acanthosis or necrosis. Slight erythema and minimal inflammation were reported when 1%, 5%, and 10% concentrations of PEG-20 Sorbitan Laurate (in water, petrolatum, or a hydrophilic ointment) were tested. Topical application of 100% PEG-20 Sorbitan Palmitate did not cause signs of irritation to the skin of rabbits during a primary skin irritation assay. PEG-20 Sorbitan Stearate (4%–100%) caused no irritation to mild irritation (PII = 0.50/4.0). PEG-20 Sorbitan Oleate (100%) was not an irritant in one study, and was a minimal irritant (PII = 0.17/4.0) in another. During a 28-day percutaneous toxicity study using rabbits, a body lotion containing 4% PEG-20 Sorbitan Palmitate (doses = 0.3 or 0.9 ml/kg/cm^2) caused mild to moderate erythema and edema, as well as scaly desquamation. A cream formulation containing 2.5% PEG-20 Sorbitan Oleate (doses = 6 mg/cm^2) caused moderate edema and erythema, slight to moderate desquamation, and mild dermatitis after 90 consecutive days of treatment during a second percutaneous toxicity study using rabbits. In a 93-day study, the cream formulation (doses = 0.36 ml/260 cm^2/3 kg rabbit) produced slight erythema and scaly desquamation. In Magnusson-Kligman guinea pig maximization tests, PEG-20 Sorbitan Laurate caused moderate to strong sensitization. In this study, the induction concentrations were 5% to 7.5%, and the challenge concentration was 100%. In studies using guinea pigs PEG-20 Sorbitan Tristearate and PEG-20 Sorbitan Oleate were not sensitizers (Elder 1984).

DeLeo et al. (1989) evaluated the cutaneous primary irritancy of several surfactants, including PEG-20 Sorbitan Laurate, using the guinea pig dermal irritation assay, and the in vitro choline release assay. The latter assay determined the effect of the surfactants on membrane choline phospholipid metabolism in human epidermal keratinocytes. The investigators hypothesized that surfactants stimulated phospholipase activation of human keratinocytes during surfactant-skin interaction, leading to the production of membrane-derived mediators, resulting in skin irritation.

The guinea pig primary dermal irritation test was a modification of the rabbit primary irritation procedure and used adult Hartley guinea pigs of both sexes (500–700 g) instead of rabbits. Feed and water were available ad libitum. The test solution (undiluted; 1ml) was applied to the shaved abdominal region using a Webril pad that was affixed with surgical tape. Adhesive tape served as an overwrap. After 4 hours, the occlusive patch was removed. This procedure was repeated for 2 more consecutive days. On the sixth day, the guinea pigs were depilated, rinsed, towel-dried, and recaged. The test sites were evaluated 4 hours later. PEG-20 Sorbitan Laurate had a score of <1 (no irritation to very slight erythema).

Human keratinocytes were incubated with [^{3}H]choline for 24 hours in the choline release assay. The keratinocytes incorporated 40% to 60% of the radioactivity of the media. The concentrations of PEG-20 Sorbitan Laurate tested were 1–4 × 10^{-4} M. Cellular extracts were examined by high performance liquid chromatography (HPLC). To determine the origin of the [^{3}H]choline released into the media by surfactant treatment, the acid-soluble and acid-precipitable pools of radioactivity were determined in surfactant-treated and control (distilled water) cultures, and the treated and control membrane phospholipids were extracted and separated by HPLC. PEG-20 Sorbitan Laurate induced choline release only at 4 × 10^{-4} M, and was classified as a minimal dermal irritant (DeLeo et al. 1989).

Gajjar and Benford (1987) used a differentiating keratinocyte cell line developed from explant cultures of rat sublingual epithelium as a model for topical skin irritancy. The investigators tested a number of surfactants, including PEG-20 Sorbitan Laurate, PEG-20 Sorbitan Palmitate, PEG-20 Sorbitan Stearate, PEG-20 Sorbitan Oleate, and PEG-20 Sorbitan Trioleate. The end points used to assess toxicity were acid phosphatase (AP) activity after 4 hours of dosing, NR uptake (see General Biology—Cytotoxic Effects), and kenacid blue (KB) staining after 3 days to assess cell viability and number. No peak in AP activity was observed after treatment with the Polysorbates, with the exception of a slight peak observed after treatment with the highest concentration (1.0 mg/ml) of PEG-20 Sorbitan Laurate; the peak was 158% that of the control. The NR-50 values for the Polysorbates were 0.59, 0.21, 0.34, 1.0, and >1.0 mg/ml for PEG-20 Sorbitan Laurate, PEG-20 Sorbitan Palmitate, PEG-20 Sorbitan Stearate, PEG-20 Sorbitan Oleate, and PEG-20 Sorbitan Trioleate, respectively. The KB-50 for PEG-20 Sorbitan Laurate was 0.44 mg/ml, the KB-50 for PEG-20 Sorbitan Palmitate was 0.23 mg/ml, the value for PEG-20 Sorbitan Stearate was 0.32 mg/ ml, the value for PEG-20 Sorbitan Oleate was 0.89,

and the KB-50 for PEG-20 Sorbitan Trioleate was > 1.0 mg/ml. Of the compounds evaluated in this study, the Polysorbates were the least toxic.

PEG-20 Sorbitan Oleate at a concentration of 50% in ethanol was tested for sensitization potential in a mouse ear sensitization assay (Descotes 1988). The test compound was applied twice to the right external ear of Swiss mice using a scapular subcutaneous injection of complete Freund's adjuvant. The degree of contact hypersensitivity was determined from left external ear swelling, calculated as the difference in external ear thickness measured immediately before challenge and 24 hours later. The external ear thickness measurements taken before and after application of PEG-20 Sorbitan Oleate did not differ significantly, indicating that the Polysorbate was not a skin sensitizer under the conditions of this study.

PEG-20 Sorbitan Oleate was not a sensitizer and did not cause swelling of the external ear in a similar study using mice (Gad et al. 1986).

Sorbitan 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 (Repeated Insult Patch Test (RIPT); comedogenicity)" and in the chorioallantoic membrane vascular assay, of which additional details were unavailable (CTFA 1998d).

Sorbitan Isostearate (2.5%) was tested in an RIPT using 201 subjects. During the induction period, 48- to 72-hour occulsive 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 1998d).

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 PIIs 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 each test substance (10.0% in squalene) 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 Esters are minimal to mild irritants. In acute skin irritation tests using rabbits, Sorbitan Stearate was mildly irritating and caused dose-dependent erythema and edema. The rabbit dermal toxicity and irritation potential of Sorbitan Sesquioleate was minimal. Sorbitan Oleate was minimally irritating to rabbit skin, and caused erythema and edema. Sorbitan Palmitate was nonirritating and did not cause systemic toxicity during a short-term dermal toxicity study. Sorbitan Tristearate was nonirritating when applied to the skin of rabbits. In rabbits, Sorbitan Trioleate was generally found to be a skin irritant; it caused erythema, edema, and thickening, but not systemic toxicity. After 3 days of treatment, Sorbitan Laurate at a concentration of 100% caused intense erythema and edema to the clipped skin of New Zealand rabbits; 10% and 60% concentrations resulted in erythema and edema. No visible change was observed after treatment with 1% Sorbitan Laurate. After 10 days of application, thickening of the skin sites was observed at 60% and 100%, and erythema and edema were observed at 1% and 10% (Elder 1985).

Polyethylene Glycol

In skin irritation tests, undiluted PEG-6 was applied to the skin of rabbits for 4 hours and 50% PEG-75 was applied to guinea pigs for 4 days and to rabbits over a 13-week period. In the guinea pig skin sensitization test, PEG-75 was tested at a concentration of 0.1%. The PEGs were not irritating to the skin of rabbits or guinea pigs, and PEG-75 was not a sensitizer (Andersen 1993).

Ocular Irritation

PEGs Sorbitan/Sorbitol Fatty Acid Esters

A series of Draize ocular irritation assays was performed on the PEGs Sorbitan/Sorbitol Fatty Acid Esters. Conjunctival irritation was observed at 1 hour, but not 96 hours after instillation of PEG-40 Sorbitan Lanolate at a concentration of 100%. PEG-30 Sorbitol Tetraoleate Laurate caused slight redness in one eye at 1 hour, but not 24 hours after instillation. The Draize scores for both compounds were 0.33/100, and both were classified as nonirritating to the eyes of rabbits. PEG-40 Sorbitan Peroleate (100% and 10% in water) and PEG-50 Sorbitol Hexaoleate (100%) were nonirritating to the eyes of rabbits. PEG-80 Sorbitan Palmitate (100%) was minimally irritating in unrinsed eyes (CTFA 1998b).

Polysorbates

Undiluted PEGs-4 and -20 Sorbitan Laurate were nonirritating to the eyes of rabbits. PEG-20 Sorbitan Laurate at a

concentration of 30% (in distilled water) was not an ocular irritant. PEG-20 Sorbitan Palmitate (30–100%), PEG-20 Sorbitan Tristearate (30%), PEG-5 Sorbitan Oleate (100%), PEG-20 Sorbitan Stearate (30%), and PEG-20 Sorbitan Trioleate (10%–75%) were not irritating to the eyes of rabbits. At a concentration of 100%, PEG-20 Sorbitan Stearate and PEG-20 Sorbitan Oleate caused either no or minimal irritation. PEG-4 Sorbitan Stearate at a concentration of 60% was minimally irritating to the eyes of rabbits (Elder 1984).

The concentrations of PEG-20 Sorbitan Laurate, PEG-20 Sorbitan Palmitate, and PEG-20 Sorbitan Oleate which caused a 50% reduction in cell viability using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay were 219, 227, and 270 μg/ml, respectively. The maximum concentrations (w/v) of these compounds that did not cause irritation to the eyes of rabbits were 16%, 41%, and 62%, respectively (Nagami and Maki 1993).

Undiluted PEG-20 Sorbitan Laurate, PEG-20 Sorbitan Palmitate, and PEG-20 Sorbitan Oleate were evaluated for ocular irritation using the in vitro Skin^2 tissue system model ZK1200. The end point of this assay was MTT reduction, which determined cell viability after exposure to the test chemicals. The times of exposure that resulted in the death of 50% of the treated cells were 9.4, >10, and >10 minutes, respectively. On the basis of this test, PEG-20 Sorbitan Laurate was classified as a mild-moderate irritant, and PEG-20 Sorbitan Palmitate and PEG-20 Sorbitan Oleate were classified as innocuous chemicals (Rachui et al. 1994). PEG-20 Sorbitan Oleate at a dose of 150 mg was a mild ocular irritant to the eyes of rabbits (Radian Corporation 1991).

North-Root et al. (1992) reported that the concentrations of PEG-20 Sorbitan Palmitate and PEG-20 Sorbitan Stearate that resulted in 50% relative survival of SIRC rabbit corneal cells were 11,482 and 36,000 ppm, respectively. A Draize assay was also performed using New Zealand white rabbits (test concentrations did not exceed 30%). The surfactant concentrations predicted to cause Draize scores of 20 were >90% and ≫90% for PEG-20 Sorbitan Palmitate and PEG-20 Sorbitan Stearate, respectively.

The maximum average scores for PEG-20 Sorbitan Oleate and PEG-20 Sorbitan Laurate in the Draize ocular irritation assay were 3.8 and 5.7, respectively (Roguet et al. 1994). PEG-20 Sorbitan Oleate at a concentration of 10% in water was also nonirritating in a Draize test using six New Zealand white rabbits (Guido 1987). The maximum Draize score for 98% PEG-20 Sorbitan Oleate in another study was 4.0/110 (Bagley et al. 1992). Jacobs and Martens (1989) reported that the mean erythema score of PEG-20 Sorbitan Oleate was 0.55, and no signs of chemosis, corneal opacity, or corneal swelling were observed in the three rabbits tested.

PEG-20 Sorbitan Laurate, PEG-20 Sorbitan Stearate, PEG-20 Sorbitan Trioleate, and PEG-20 Sorbitan Oleate were classified as nontoxic to the eye in a study using the permeability of the mouse cornea as a test for acute ocular toxicity (Maurice and Brooks 1995). In this study, a drop of the test compound was placed on the cornea for 1 minute and was washed off and replaced with a drop of sulforhodamine B, a fluorophore dye. Permeability was determined by the total fluorescence of the cornea. The investigators noted the potential of PEG-20 Sorbitan Laurate to increase the penetration of the dye.

In a similar study, 1% PEG-20 Sorbitan Oleate did not significantly increase the permeability of the mouse cornea to fluorescein (Etter and Wildhaber 1985). PEG-20 Sorbitan Oleate at a concentration of 100% did not significantly increase the opacity or permeability of the bovine cornea (six per test compound) to sodium fluorescein (Vanparys et al. 1993).

The red blood cell (RBC) assay estimates the irritation potentials of surfactants and detergents by measuring the photometrical absorbance of oxyhemoglobin, an indicator for cell membrane lysis and cell protein denaturation. When tested using this assay, PEG-20 Sorbitan Laurate did not produce hemolysis and the denaturation index (equal to 100% at a concentration of 30 moles sodium dodecyl sulfate per mole oxyhemoglobin as an internal standard) was <1 (Pape, Pfannenbecker, and Hoppe 1987).

Tachon et al. (1989) compared the in vitro cellular toxicity and the in vivo ocular irritation potency of 16 surfactants, including PEG-20 Sorbitan Laurate. Cellular toxicity was estimated in V79 Chinese hamster lung fibroblasts using a cell mortality test and a cell growth inhibition assay. In the cell mortality test, V79 cells were suspended in Eagle's modified minimal essential medium (EMEM) for 1 hour at 37°C, and various concentrations of the surfactants were added. The treatment solution was discarded and the cells were resuspended in fresh medium to which 25 μl of trypan blue had been added. Cell mortality was assessed in terms of membrane integrity; trypan blue was excluded by intact cells, but penetrated membrane-damaged cells. The lethal concentration 50% (LC_{50}) of PEG-20 Sorbitan Laurate was 98.34 μg/ml. When 10% fetal calf serum was added to the medium, the LC_{50} increased to 595.30 μg/ml, indicating that the surfactant interacted with serum proteins. In the cell growth inhibition test, V79 cells were incubated in well plates with EMEM and 10% fetal calf serum to which the surfactants had been added. After 72 hours, phase contrast inverted microscopy was used to evaluate cellular morphology changes. Cell growth was assessed by measuring the total protein content per well, with bovine serum albumin as a standard. The PEG-20 Sorbitan Laurate concentration required to reduce growth by 50% was 287.63 μg/ml, as determined using the cell growth inhibition test. This assay did not discriminate between cell death and inhibition of growth, however.

PEG-20 Sorbitan Laurate at a concentration of 10% in water was a very weak ocular irritant in the Draize test using six albino rabbits. The maximum ocular irritation score was 5.67 after 1 or 24 hours, and the irritation score was zero after 7 days. The investigators concluded that the results of the Draize test correlated with the results of the two previous in vitro assays (Tachon et al. 1989).

Isolated rabbit eyes were used in an ocular toxicity assay as a model for human corneal damage after exposure to various

chemicals, including PEG-20 Sorbitan Laurate. The eyes were enucleated within 30 minutes of death and were stored at 4°C overnight. The enucleated globes were kept in a temperature-control chamber (32–36°C), held vertically in clamps, and irrigated with Hanks' balanced salt solution at the upper limbus. The globes were examined using a Haag-Streit Slit Lamp before and after treatment, and the corneal thickness was measured. After 90 minutes, 20- and 100-μl volumes of PEG-20 Sorbitan Laurate were applied at the superior limbus for 10 seconds and 1 minute, respectively. The larger volume was applied in 20-μl aliquots at 10-second intervals. The globes were rinsed for 10 seconds with the salt solution. The corneal thickness was measured every 30 minutes; the total time of the experiment was 7 to 8 hours. Exposure to PEG-20 Sorbitan Laurate had little effect on corneal deturgescence in enucleated rabbit globes, compared to controls. Increased granularity of the epithelium and fluorescein staining in the contact area were observed after treatment with PEG-20 Sorbitan Laurate (Berry and Easty 1993).

Surfactant cytotoxicity was evaluated in primary cultures of rabbit corneal epithelial cells by Grant et al. (1992) and Yao and Acosta (1992) using as end points lactate dehydrogenase (LDH) enzyme leakage from the cytosol to the medium, mitochondrial MTT dye reduction, and lysosomal NR uptake, followed by morphological observations at 1 and 24 hours after treatment. The LDH leakage assay evaluated cell membrane integrity, and the MTT reduction and NR uptake assays determined cell viability (Grant et al. 1992; Yao and Acosta 1992). In the study by Grant et al. (1992), cells treated with PEG-20 Sorbitan Laurate had marked vacuolization with little formation of pseudopodia. Most LDH leakage occurred during the 1-hour treatment time, and was <20% after 24 hours. In the Yao and Acosta study (1992), treated cells had either a pleomorphic epithelial appearance or a keratinocyte-like appearance. Of the surfactants tested (including benzalkonium chloride and sodium dodecyl sulfate [SDS]), PEG-20 Sorbitan Laurate was the least cytotoxic in both studies. The in vitro results were correlated with Draize rabbit ocular irritation data in which PEG-20 Sorbitan Laurate was practically nonirritating to the eyes of rabbits (Grant et al. 1992; Yao and Acosta 1992).

Yang and Acosta (1994) determined cytotoxicity of surfactant mixtures by LDH leakage and MTT reduction in a primary culture system of rabbit corneal epithelial cells. The investigators then correlated the in vitro cytotoxicity with reported Draize ocular irritation data. Binary and tertiary groups of surfactant mixtures were tested. The total active surfactant concentration of both groups was 7%. The binary group contained lauramphocarboxyglycinate (HMS) and SDS in varying proportions, and the ternary group contained HMS, SDS, and 8% PEG-20 Sorbitan Laurate. At low and high surfactant concentrations, LDH leakage increased by <20% compared to controls. A 20% to 50% increase occurred at the middle concentrations. A similar pattern occurred in the MTT reduction assay. The ternary surfactant mixture containing PEG-20 Sorbitan Laurate was slightly more cytotoxic than the binary mixture, although undiluted PEG-20 Sorbitan Laurate was nonirritating to the rabbit eye. The investigators speculated that PEG-20 Sorbitan Laurate affected the toxicity of other surfactants by facilitating the uptake of SDS and HMS (by decreasing the surface tension of the plasma membrane) or by changing the micellar organization of hydrophilic/lipophilic balance ratios of surfactants.

PEG-20 Sorbitan Palmitate was minimally irritating to the eyes of guinea pigs in the Draize ocular irritation assay when tested at a concentration of 5% (w/v); the Draize score was 4.4. In vitro assays using mouse embryo fibroblasts (BALB/c 3T3 cells; NR assay only) and BHK 21/C13 cells were also performed; PEG-20 Sorbitan Palmitate was minimally irritating (Bracher et al. 1988). The NR-50 score was 269.3 μg/ml, the concentration that caused 25% cell detachment was 750 μg/ml, the concentration that caused 50% growth inhibition was 115 μg/ml, the concentration that caused fluorescein retention (fluorescence shift, FS-25) was 5.6 μg/ml, and the concentration that resulted in a 25% viability ratio based upon ethidium bromide exclusion was 21 g/ml.

In other in vitro assays, PEG-20 Sorbitan Laurate and PEG-20 Sorbitan Oleate at concentrations of 5% to 10% (w/v) increased opacity and thickness of isolated bovine cornea after 30 minutes to 4.5 hours of incubation (Igarashi and Northover 1987).

Polyethylene Glycol

PEG-6 and -75 did not cause corneal injuries when instilled (undiluted, 0.5 ml) into the conjunctival sac of rabbits. PEG-8 (35% solution, 0.1 ml) and PEG-32 (melted in water bath, 0.1 ml) induced mild ocular irritation in rabbits (Andersen 1993).

Sorbitan Esters

Sorbitan Isostearate was nonirritating to the eyes of rabbits in 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).

Sorbitan Stearate was not an ocular irritant in a study using rabbits when the ester was tested at high concentrations. Low concentrations in formulation caused slight conjunctival irritation. High concentrations of Sorbitan Sesquioleate were nonirritating. One study with Sorbitan Laurate and two each with Sorbitan Oleate, Sorbitan Tristearate, and Sorbitan Palmitate were negative for ocular irritation in the rabbit. Concentrations of 30% to 100% Sorbitan Laurate were nonirritating to the eyes of rabbits in Draize ocular irritation tests (Elder 1985).

Comedogenicity

When applied once daily, five times weekly to the external ear of the New Zealand white rabbit for 3 weeks, a 1% aqueous solution of PEG-20 Sorbitan Laurate had no comedogenic activity (Morris and Kwan 1983).

A product containing 5% Sorbitan Isostearate was tested to determine its comedogenicity potential in 20 human subjects. Reactions that scored a value of one 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 ($p \leq 0.05$). 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 0.05. It was concluded that this product did not produce lesions of comedogenicity (CTFA 1998d).

Inhalation Toxicity

Martinez and Brown (1991) evaluated the pulmonary toxicity in male Sprague-Dawley rats of 7% PEG-20 Sorbitan Oleate in comparison with the toxicities of a herbicide and 7% polyoxyethyleneamine (POEA). The rats were anesthetized prior to administration of 0.1, 0.2, and 0.4 ml doses of the test compounds directly into the trachea. The negative control was saline. The five rats per group were observed for 1 hours for signs of respiratory distress or early death. After 24 hours, the rats were killed for necropsy. The lungs were removed immediately after death, dissected free from other structures, blotted, and evaluated. Lung weight measurements and a subjective scaling system were used to evaluate the severity of damage. Each lung was given a value of 0 to 5, with 5 being hemorrhages involving the whole lung.

In this study, treatment with 0.1 to 0.2 ml of PEG-20 Sorbitan Oleate did not result in deaths. Of the rats of the high dose group, 30% died. The negative control, saline, killed 20% of rats given the 0.2 ml dose, but none of the rats given the high or low doses. PEG-20 Sorbitan Oleate had no effect on lung weight at 0.1 and 0.2 ml, but the weight was increased from 1.4 to 1.8 g at the high dose. It did not increase lung damage at 0.1 to 0.2 ml, but increased the score to 1.3 ("little obvious dysfunction") at the high dose. The negative control scores were 0.25, 0.1, and 0.4 for 0.1 to 0.4 ml, respectively. It was concluded that PEG-20 Sorbitan Oleate had few significant pulmonary effects except at the highest dose.

The plasticizer di(2-ethylhexyl)phthalate (DEHP; 125–300 mg/kg) caused acute lung injury in adult male rats after it was injected intravenously with 13.3% PEG-20 Sorbitan Oleate and 0.9% saline (Schulz 1974). Labored respiration and cyanosis occurred within minutes of the injection of the high dose, and 80% of the rats died within 3 hours of treatment, but none thereafter. The lungs were grossly enlarged and darkened. After injection of 200 mg/kg DEHP, the wet lung to body weight ratio increased compared to that of controls, but the wet lung to dry lung weight ratio did not differ, suggesting the presence of excess proteinaceous material in the lung. Rats given 125 mg/kg had significant polymorphonuclear leukocytic infiltration of the interalveolar septa. The vehicle itself had no effect on lung weight when compared with non-injected controls. Similarly solubilized preparations of corn oil or di(2-ethylhexyl)sebecate, and solutions consisting of up to 500 mg/kg DEHP in bovine serum albumin or gum arabic did not produce acute lung effects. The investigator concluded that a specific interaction between DEHP and PEG-20 Sorbitan Oleate had occurred to produce the observed lesions.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Polysorbates

PEG-20 Sorbitan Laurate was used in teratologic studies as a solubilizer for poorly soluble substances such as thalidomide and its metabolites. In these studies using Swiss mice, the PEG-20 Sorbitan Laurate vehicle had no effect on developing embryos when given doses of 10 ml/kg in saline (Meise, Ockenfeis, and Köhler 1973; Köhler and Koch 1974; Fickentscher et al. 1977). During studies using CBA and C57BL mice, more than 50% of the mice died after being given 2.5 ml/kg IP doses of the PEG-20 Sorbitan Laurate vehicle in saline (Kocher-Becker and Kocher 1981). Scott, Fradkin, and Wilson (1977) reported similar results in IP studies using thalidomide in PEG-20 Sorbitan Laurate. When 40 ml/kg of the vehicle alone was administered, five of five nonpregnant Harlan (ICR-derived) mice died. A test volume of 20 ml/kg killed three of five nonpregnant mice and five of five nonpregnant Royalhart (Wistar-derived) rats. No deaths occurred after administration of 10 ml/kg, but hepatic swelling was observed at necropsy. In mice, 200 mg/kg of the vehicle produced 10.1% dead or resorbed embryos, and 0.8% malformations in survivors out of 139 total implants. Without treatment, 2.5% of the 120 total implants was dead or resorbed and 0.9% of the survivors was malformed. No difference in the mean fetal weight was observed. Pregnant rats given 400 mg/kg of the vehicle had 160 total implants; 7.5% was dead or resorbed, and 0.7% of the survivors was malformed. Untreated rats had 666 total implants, of which 5% was dead or resorbed, and 1% of the survivors was malformed. No difference in fetal weight was observed (Scott, Fradkin, and Wilson 1977).

Six pregnant NMRI mice and 11 pregnant Swiss mice received a single IP injection of PEG-20 Sorbitan Laurate on the ninth day of gestation (Kocher-Becker and Kocher 1981). The PEG-20 Sorbitan Laurate doses were 1.0, 1.7, 2.5, 3.3, and 5.0 ml/kg. The doses were diluted to 10 ml/kg or 20 ml/kg (high-dose only) with physiological saline. For the NMRI mice, five of six dams died after receiving 5.0 ml/kg PEG-20 Sorbitan Laurate. Three of 12 NMRI dams died, and a further 3 NMRI mice aborted after receiving 3.3 ml/kg PEG-20 Sorbitan Laurate. In Swiss mice, 9 of 11 dams died after receiving the high dose. All pregnancies were terminated on day 16 or 18 of gestation. The fetuses were inspected for gross malformations and stained for examination of the skeleton (Table 7). All doses of PEG-20 Sorbitan Laurate caused malformations in offspring of both treated strains. In Swiss mice, the number of malformed fetuses was dose-dependent, but the number of litters with malformed fetuses was not. The same applied to the results using NMRI mice, but only when the 2.5 ml/kg dose was compared with the lower two doses. The percentage of malformed fetuses was greater in the Swiss strain than in the NMRI strain. Malformations observed included wedge-shaped and incomplete vertebrae,

TABLE 7
Reproductive and developmental toxicity of PEG-20 Sorbitan Laurate (Kocher-Becker and Kocher 1981)

	Mouse strain							
	NMRI dose (ml/kg)					Swiss dose (ml/kg)		
	1.0	1.7	2.5	3.3	Control	1.0	2.5	Control
Maternal/litter information								
Dams	7	10	35	12	25	15	21	8
Maternal deaths	0	0	2	3	0	1	4	0
Litters aborted	0	0	2	3	0	0	1	0
Litters with at least one malformed fetus	2	2	17	2	2*	7	9	0
Implantations	83	118	361	79	278	192	237	141
Individual fetus information								
Resorbed	6	11	71	23	18	23	55	8
Percent resorbed	7.2	9.3	19.7	29.1	6.5	12.0	23.2	5.7
Dead	0	0	3	0	1	0	6	3
Living	77	107	287	56	259	169	176	130
Malformed (living)	2	4	41	6	2*	20	57	0
Percent malformed (living)	2.6	3.7	14.3	10.7	0.8	11.8	32.4	0
Types of malformations								
Limbs alone or combined with others	0	0	6	0	0	3	4	0
Vertebrae and ribs without limb involvement	1	4	33	6	0	17	53	0
Others	1	0	2	0	2*	0	0	0

*Atypical: exencephaly

fused vertebral arches in the thoracic and lumbar region, and/or rib fusions of varying severity in length of fusion and numbers of ribs involved. Limb malformations, including phocomelia, occurred sporadically at all doses, and were mainly of the thalidomide type.

Wickramaratne (1987) evaluated the teratogenicity of PEG-20 Sorbitan Laurate and other chemicals in Alpk:AP (Wistar-derived) rats using the Chernoff-Kavlock assay. Dams (15 per group) were treated with 10 ml/kg/day PEG-20 Sorbitan Laurate at varying concentrations on gestational days (GD) 7 to 17. Maternal body weights were determined on GD 1, 7 to 17, and 22. Offspring were weighted on days 1 and 5 postpartum and the numbers of live and dead pups were counted. No specific examination for malformations was performed. The control was physiological saline. PEG-20 Sorbitan Laurate reduced offspring litter size, survival, and weight gain when the dams were given the chemical intraperitoneally, but the parameters did not differ significantly from controls when the Polysorbate was administered orally, dermally, or subcutaneously (Table 8).

PEG-20 Sorbitan Laurate, PEG-20 Sorbitan Palmitate, PEG-20 Sorbitan Stearate, and PEG-20 Sorbitan Oleate caused serious developmental effects in sea urchin embryos when administered at concentrations as low as 0.004% in artificial sea water. At concentrations >0.1%, the Polysorbates blocked cell cleavage and lysed two-cell stage embryos. The investigators noted, however, that the sea urchin embryo model was more sensitive than those using mammalian cells in culture (Bresch and Ockenfels 1977).

The NTP (1992a, 1992c) studied time-mated Sprague-Dawley female rats (8–10-week-old) given 5 ml/kg/day of aqueous PEG-20 Sorbitan Laurate by gavage on GD 6 to 15. The treatment doses were 500 (25 rats) and 5000 mg/kg/day (24 rats). The body weights were recorded at GD 0, 3, 6 to 15, 18, and 20. Feed and water consumption were monitored throughout the study. The dams were killed on GD 20. Pregnancy status was determined by uterine examination. The body, liver, right kidney, heart, and uterus of each rat were weighed and fixed for microscopic examination. Live fetuses were dissected from the uterus, weighed, and examined for external abnormalities and visceral malformations.

All treated female rats survived to scheduled necropsy, and 22 to 24 pregnancies per group were confirmed. Treated dams had transient weight loss (>5 g/dam) between GD 6 and 7; 3/24 dams of the low-dosing group and 7/22 of the high-dosing group experienced weight loss, compared to 1 of the 22 untreated control rats. By GD 20, alopecia (regional hair loss) occurred in 5/24 and 5/22 rats of the low- and high-dosing groups, respectively. No untreated rats had hair loss. No other signs of toxicity were observed, and average maternal body weights did not differ between groups. No treatment-related change in maternal weight gain occurred during gestation. Maternal weight gain during treatment was decreased by 14% at 5000 mg/kg/day relative to that of the vehicle control, but no effect was observed at 500 mg/kg/day. Maternal organ weights and feed intake did not differ between groups. Maternal water intake was increased by 14%

TABLE 8
Reproductive toxicity of PEG-20 Sorbitan Laurate using rats (Wickramaratne 1987)

Concentration, dose, route	Group mean weight change (GD 1–22)	Group mean weight change (GD 7–16)	No. pregnant rats	No. viable litters		Mean litter size (live + dead)
				Day 1	Day 5	
10%, 10 ml/kg, oral	121.3 ± 15.3	105.0 ± 12.6	15	15	14	12.1 ± 2.51
Oral control[a]	115.6 ± 15.5	95.1 ± 29.0	15	15	15	10.7 ± 3.94
10%, 10 ml/kg, IP	68.5 ± 24.5	41.0 ± 29.0	8	6	4	6.2 ± 3.76
IP control	111.9 ± 15.6	85.9 ± 16.6	12	11	11	7.6 ± 3.57
100%, 2 ml/kg, demal[b]	89.6 ± 14.3	61.9 ± 11.3	15	15	15	11.0 ± 3.49
Dermal control	96.1 ± 11.3	67.1 ± 11.4	15	15	15	13.2 ± 2.24
50%, subcutaneous	95.6 ± 14.7	76.7 ± 9.7	15	15	15	10.6 ± 3.02
100%, subcutaneous	108.7 ± 13.2	69.6 ± 8.2	14	14	13	12.2 ± 2.64
Subcutaneous control	115.9 ± 21.0	77.2 ± 15.1	15	15	15	10.8 ± 2.78

[a]Control and vehicle was physiological saline.
[b]6 h occlusive dressing on GD 7–17.
GD = gestational day.

from GD 6 to 15 at 5000 mg/kg/day compared to the control. The maternal low effect adverse effect level was 5000 mg/kg/day (based on the decrease in weight gain), and the maternal no-observed-adverse-effect level (NOAEL) was 500 mg/kg/day.

No differences between groups occurred in the number of corpora lutea per dam, the number of implantation sites per dam, or the percent preimplantation loss per litter. The incidence of resorptions per litter was significantly lower ($p < 0.05$) in the 5000 mg/kg/day dosing group compared to the both the vehicle control group and historical controls. Resorption incidences were 4.1% (control), 4.2% (500 mg/kg/day), and 0.9% (5000 mg/kg/day), respectively. No significant effects were observed for the percent litters with at least one resorption. No late fetal deaths occurred, and no litters with 100% prenatal mortality were observed. PEG-20 Sorbitan Laurate exposure had no effect on live litter size, percent males per litter, fetal body weight, percent adversely affected fetuses per litter, or percent litters with one or more resorbed or adversely affected implants. The percent fetuses per litter with external or visceral malformations and the overall incidence of malformations did not differ among the groups. Only two fetuses (of 308) of the 5000 mg/kg/day–treated dams had skeletal malformations; these malformations (bipartite vertebra in the thoracic region) were, however, common in Sprague-Dawley rats. PEG-20 Sorbitan Laurate had no adverse effects on the growth, viability, or morphological developments of fetuses of treated dams. The developmental NOAEL was >5000 mg/kg/day (NTP 1992a; 1992c).

PEG-20 Sorbitan Stearate and PEG-20 Sorbitan Laurate were fed to C57BL/6 mice at a concentration of 10% during a three-generation study. The first generation consisted of seven breeding groups: a control group, groups in which both sexes were fed a Polysorbate diet, and groups in which one sex was fed a Polysorbate diet and the other was fed a control diet. Only groups in which both sexes were fed the same diet were continued for the second and third generations. Four litters of mice were delivered from "brother-sister" matings by the first and second generation mice and three litters by the third generation. Offspring of dams that were fed one of the Polysorbate diets had significnalty decreased weaning weights when compared to offspring of dams fed the control diet. The decrease was due to a poorer weight gain in the experimental offspring during the third week of life. Mice fed PEG-20 Sorbitan Stearate had significantly smaller litters than mice fed the control diet, and dams fed PEG-20 Sorbitan Stearate delivered significantly more offspring dead at birth than mice fed the control PEG-20 Sorbitan Laurate diets. Females fed the experimental diets produced their largest litter at a later age than females fed the control diet. Feeding of the Polysorbates did not have any significant effect upon the weight of adult mice, the weight of gonads of adult mice, or the rate of sperm movement. Other details were unavailable (Paschall 1964).

Negative results were reported when the teratogenicity of PEG-20 Sorbitan Oleate was evaluated using the whole-embryo culture. Schmid, Trippmacher, and Bianchi (1998) cultured Han Wistar rat embryos in 5 ml of homologous, undiluted rat serum and incubated them for 48 hours. The test chemicals were added to the medium after dilution with distilled water or suspension in 2% gelatin. The system also contained S9 mix for the entire culture period. When heart beat and flexion were present, and at least 50% of the embryos had established functional yolk sac-blood circulation, the embryos were evaluated for signs of teratogenicity. Embryos classified as abnormal had deviations from the controls and had crown-rump lengths that were at least 80% of the mean control value.

In a study using neonatal female rats (Gadjová, Jakuborshy, and Války 1993), PEG-20 Sorbitan Oleate accelerated maturation, prolonged the estrous cycle, and induced persistent vaginal estrous when injected IP on days 4 to 7 after birth. Six rats were

injected with 1% PEG-20 Sorbitan Oleate, five were injected with 5%, and six were injected with 10%; the injection volume was 0.1 ml/rat. The untreated control group included 8 rats, the negative-control group (aqua pro injection) included 10 rats, and the positive-control group (diethylstilboestrolum in oil helianthus) contained 5 rats. The estrous cycle was evaluated at weeks 10, 14, and 18, and vaginal smears were obtained daily for 14 days. The rats were killed at 5 months of age, and the uterus, ovaries, adrenal glands, and pituitary gland were removed, weighed, and examined. PEG-20 Sorbitan Oleate did not affect growth or viability of the treated rats. A significant decrease in body weight was observed in rats of the 1% dose group as compared to the control group. All rats given the Polysorbate had significantly advanced vaginal opening in comparison with controls. The average length of the estrous cycle was 9.3 to 14 days in rats of the treatment groups. For rats of the untreated and positive control groups, the lengths were 4.3 and 9.4 days, respectively. The ovaries of high-dose rats had multiple cavities (3 mm diameter) in three rats; two rats of the positive-control group had similar lesions. An enlarged uterus was observed in two rats of the low-dose group. The relative weight of the adrenal glands was increased in all treatment groups compared to the untreated control group, but the increase was significant only in the low-dose group ($p \leq 0.05$). The relative weight of the ovaries was decreased significantly in all PEG-20 Sorbitan Oleate-treated groups when compared to the untreated control. The uterine weight was decreased significantly in rats treated with 5% PEG-20 Sorbitan Oleate and in rats of the positive-control group. Microscopic findings in rats of the treatment groups were similar to those of the positive-control group. In the uterus, squamous cell metaplasia of the epithelium and other cytological changes that were indicative of chronic estrogenic stimulation were observed. The ovaries were without corpora lutea, and had degenerative follicles.

PEG-20 Sorbitan Oleate (2500 mg/kg/day) did not cause developmental toxicity in the CD-1 mice during an in vivo teratology screening study in which pregnant mice were treated by gavage on GD 8 to 12 (Kavlock, Short, and Chernoff 1987).

The teratogenicity of PEG-20 Sorbitan Stearate was evaluated by Ema et al. (1988) using pregnant Wistar rats. The rats were fed 0.1%, 1.0%, or 10% of the Polysorbate from GD 7 to 14. Rats of the low dose group consumed 99 mg/kg/day, rats of the medium dose group consumed 960 mg/kg/day, and rats of the high dose group consumed 7693 mg/kg/day. Under the conditions of this study, PEG-20 Sorbitan Stearate had no harmful effects on the prenatal development of the rat. Changes were not observed in the number, sex ratio, or body weight of live fetuses, and external, visceral, and skeletal malformations of the offspring were not detected.

Hardin et al. (1987) fed pregnant CD-1 mice 5200 mg/kg/day PEG-20 Sorbitan Stearate on GD 6 to 13. None of the 50 treated dams died as a result of treatment with the control diet (corn oil) or the Polysorbate. Values for the control group are stated in parentheses. The maternal weight gain was 5.0 ± 3.4 g (4.6 ± 3.4 g) and the number of viable litters was 34 of 34 (34 of 37). The number of liveborn pups per litter was 10.5 ± 2.7 (9.1 ± 3.9), the percentage survival was 98.7 ± 4.3 (98.4 ± 16.0), and the birth weight per pup was 1.5 ± 0.1 g (1.5 ± 0.3 g). The only significant finding ($p < 0.05$) was reduced birth weight or weight gain; the weight gain per pup was 0.3 ± 0.2 g (0.5 ± 0.2).

Brubaker, Tayler, and Bull (1982) evaluated the behavioral effects of PEG-20 Sorbitan Oleate in dams milk after dosing female Sprague-Dawley rats with 1.25 ml/l PEG-20 Sorbitan Oleate in drinking water. The 65-day-old rats were fed ad libitum the basal feed for 5 days. The rats were given PEG-20 Sorbitan Oleate for 14 days prior to mating. At parturition, each litter was culled to eight male pups. From 10 days after birth until weaning, each dam and her litter were placed in a home cage apparatus designed to measure the activity of the pups. Mean daily activity was measured by photocell counts. PEG-20 Sorbitan Oleate appeared to enhance locomotor activity and exploratory behavior at ages 16, 17, 18, and 20 days. Mean daily and hourly activities were significantly greater in pups exposed to PEG-20 Sorbitan Oleate than pups of the control group, but only at nighttime.

PEG-20 Sorbitan Oleate decreased the size of litters when fed to rats at doses of approximately 0.8 to 3.0 g/kg (Elder 1984).

Ethylene Glycol And Its Ethers

It is generally recognized that the PEG monomer, ethylene glycol, and certain of its monoalkyl ethers (e.g., methoxyethanol, also known as 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 (CIR 1996). 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.).

Given the methods of manufacture of the PEGs Sorbitan Fatty Acid Ester, that there is no likelihood of methoxyethanol, ethoxyethanol, etc., being present as an impurity, and that the esters are chemically different from the alkyl ethers, the Panel concluded no reproductive or developmental hazard is posed by these compounds.

Polyethylene Glycol

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

MacKensie 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 a diet containing 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 (3000–7000 mg/kg/day), HSH did not produce reproductive or developmental effects (Modderman 1993).

GENOTOXICITY

PEG-40 Sorbitan Peroleate

PEG-40 Sorbitan Peroleate was nonmutagenic in the Ames test; details of this study were not available (CTFA 1998b).

Polysorbates

PEG-20 Sorbitan Laurate was nonmutagenic in the L5178Y $TK^{+/-}$ mouse lymphoma assay with and without S9 metabolic activation (Coppinger, Brennan, and Thompson 1981).

PEG-20 Sorbitan Stearate gave positive results in the Rec-assay using *Bacillus subtilis*, but gave negative results in the Ames Test (Kada, Hirano, and Shirasu 1980).

Odashima (1976) reported results of genotoxicity assays of a number of chemicals, including PEG-20 Sorbitan Stearate and PEG-20 Sorbitan Oleate. The investigators performed the newborn test, transplacental carcinogenesis assay, chromosomal aberration assays, and mutagenicity assays in microbial systems, mammalian cells, and insects. For the chromosomal aberration studies, Chinese hamster cell line KC-1 and mouse bone marrow cells were used. During the microbial mutagenicity assays, *Salmonella* strains TA1535, TA1536, TA1537, TA1538, WP2, TA100, and TA98 were used for evaluating mutagenic activity, whereas strains H-17, M-45, W3110, and TA1978 were used for repair testing. In the other assays, mutagenicity was determined using XP cells transformed by SV40 and the silkworm oocyte system. In the transplacental study, experimental animals (number and species not available) were treated three times on GD 15, 17, and 19 or on GD 14, 16, or 18. The dose administered was approximately the maximum dose that did not cause abortion and early death of the sucklings. The observation period for tumor development was limited to 1 year after birth. For the newborn study, the neonates were given subcutaneous injections on days 1, 8, 15, and 22 after birth. The dose administered was the approximate maximum dose that did not cause early death of greater than 20% of the animals, and the observation period was limited to 1 year after birth. PEG-20 Sorbitan Stearate produced false-negatives in the chromosomal aberration, microbial mutagenicity, and mammalian or insect mutagenicity assays, and produced positive parallelism in the transplacental and newborn tests. PEG-20 Sorbitan Stearate was classified as a carcinogen. PEG-20 Sorbitan Oleate produced false-positive results in the transplacental and newborn assays, and positive parallelism was reported in tests for mammalian, insect, or microbial mutagenicity and chromosomal aberrations; therefore, PEG-20 Sorbitan Oleate was classified as a noncarcinogen. The determination of "false-negative" was based on previous studies using rodents, for which the data are unavailable. The investigators concluded in some cases that the procedures used were not practical for screeing suspected carcinogens, as frequencies up to 53% and 56% were reported for false-positives and false-negatives, respectively.

In a later study, however, PEG-20 Sorbitan Stearate was considered noncarcinogenic (Kawachi et al. 1980a). Without metabolic activation, it produced negative results in the silkworm chromosomal aberration test, hamster sister chromatid exchange assay, and mutagenicity assays using *S. typhimurium* TA100 and TA98. It was positive in the rec assay. Results were not provided for studies performed in the presence of S9. Inoue, Sunakawa, and Takayama (1980) reported that 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.

PEG-20 Sorbitan Palmitate did not cause chromosome damage in Chinese Hamster ovary cells, but the Polysorbate was cytotoxic and caused marked reductions in cell number compared with detergent-free controls (Flower, Phillips, and Andersen 1988).

PEG-20 Sorbitan Stearate and PEG-20 Sorbitan Oleate (in physiological saline) were evaluated for genotoxic potential using the Chinese hamster cell chromosomal aberration assay (Ishidate and Odashima 1977). The maximum doses tested were 0.2 and 0.1 mg/ml, respectively. Of the treated cells with each Polysorbate, 1% had chromosomal aberrations (chromatid gaps). The chemicals were classified as nonmutagenic and noncarcinogenic, with the exception of PEG-20 Sorbitan Stearate, which produced significant genotoxic effects in metabolic activation systems.

PEG-20 Sorbitan Oleate caused no evidence of mutagenicity when tested using *S. typhimurium* strains TA98, TA100,

TA1535, and TA1537, with and without metabolic activation (NTP, 1992b). It was not genotoxic in microbial systems or in mammalian systems (Kawachi et al. 1980a; 1980b); the systems used were *S. typhimurium* TA100 and TA98 (mutation), *B. subtilis* (rec assay), hamster lung fibroblasts and human embryo fibroblasts (chromosomal aberrations, sister chromatid exchanges), rat bone marrow (in vivo chromosome aberrations), and the silk worm (mutation). PEG-20 Sorbitan Oleate reduced the frequency of acridine orange-induced gene conversions in *Saccharomyces cerevisiae* D7, but did not affect the frequency of UV-induced recombinations (Arni 1985). In one study (Scott and Alderson 1973), *Aspergillus conida* grown in PEG-20 Sorbitan Oleate suspension was less susceptible (by approximately a factor of two) to lethal and mutagenic damage caused by ionizing radiation.

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

In a study examining the role of inhibition of DNA repair as a mechanism in cocarcinogenesis, Sorbitan Oleate, at a concentration of 0.01%, was found to inhibit the repair of UV-irradiated DNA extracted from normal human lymphocytes (Gaudin et al. 1971).

Sorbitan Esters

Sorbitan Stearate was not mutagenic in bacteria with or without metabolic activation, and 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).

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 indadequate 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; 1 cells 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).

An unspecified Sorbitan Fatty Acid Ester (maximum dose = 5.0 mg/plate, in DMSO) was tested for mutagenicity in the Ames test using *S. typhimurium* strains TA92, TA94, TA98, TA100, TA1535, and TA1537. In the chromosomal aberration 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 h after treatment. The results were considered equivocal, and polyploidization effects were observed (Ishidate et al. 1984).

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 mg 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).

Polyethylene Glycol

PEG-8 was negative in the Chinese hamster ovary cell mutation test and the sister chromatid exchange test; the maximum test concentration in both studies was 1%. In the unscheduled DNA synthesis assay, a statistically significant increase in radioactive thymidine incorporation into rat hepatocyte nuclei was noted only at the highest concentration tested (0.1% PEG-8). PEG-150 was not mutagenic in the mouse lymphoma forward mutation assay when tested at concentrations up to 150 g/l (Andersen 1993).

CARCINOGENICITY

Polysorbates

PEG-20 Sorbitan Oleate was evaluated for carcinogenicity in a 2-year (103-week) feed study (NTP 1992b) using F344/N rats and B6C3F$_1$ mice. Sixty animals per sex per group were given 25,000 or 50,000 ppm of PEG-20 Sorbitan Oleate in feed daily. Feed and water were available ad libitum. Clinical observations were made twice daily, and findings were recorded weekly for 13 weeks, then monthly or as necessary afterwards. After the first 15 weeks of treatment, an interim evaluation was performed in which 7 to 10 mice and rats given 0 or 50,000 ppm underwent a complete histopathological examination. At this time, no changes in relative or absolute organ weights were observed in any group as compared to controls. Female mice fed 50,000 ppm PEG-20 Sorbitan Oleate had an increased incidence of hyperplasia and inflammation of the nonglandular stomach. Neoplasms observed during this examination were not considered related to the administration of the test compound. At the end of the study, the final mean body weight of female mice fed 50,000 ppm was decreased by 11%, as compared to controls. Male rats fed the high dose had decreased survival (0 ppm,

29/50; 25,000 ppm, 18/50; 50,000 ppm, 18/50) due to neoplasms commonly observed in aging F344/N rats, including mononuclear cell leukemia, pituitary gland adenoma, preputial gland carcinoma, mammary gland fibroadenoma, Zymbal's gland carcinoma, and mesothelioma. Survival in the other groups did not differ from controls (female rats: 23/50, 25/50, 25/50; male mice: 33/49, 34/50, 32/50; female mice: 30/50, 28/50, 26/50, respectively, for 0–50, 000 ppm dose groups). No clinical findings were noted. In male rats, the incidences of benign and malignant pheochromocytoma were 21/50, 19/50, and 29/50; the incidence was significantly increased in the high-dose group, compared to the control group. For benign neoplasms, the incidences were 21/50, 16/50, and 28/50. For malignant neoplasms, the incidences were 1/50, 4/50, and 1/50. Male rats of the low-dose group had a decreased incidence of hyperplasia of the adrenal medulla, whereas the incidence in males of the high-dose group was increased compared to the control group. The incidences of hyperplasia of the adrenal medulla for male rats were 11/50, 22/50, and 12/50. For mice treated with PEG-20 Sorbitan Oleate, no increase in the incidence of neoplasms were observed. Investigators, however, observed increased incidences of squamous hyperplasia and inflammation of the nonglandular stomach in mice of the high-dose group. Female mice of the high-dose group also had an increased incidence of ulcers of the nonglandular stomach.

The investigators concluded that, based upon the increased incidence of pheochromocytomas of the adrenal medulla, "equivocal evidence of carcinogenicity" existed for the male F344/N rat (equivocal evidence = marginal increase of neoplasms that may be chemical related). No evidence of carcinogenic activity was observed in female rats or mice of either sex fed up to 50,000 ppm PEG-20 Sorbitan Oleate. The test compound was associated with inflammation and squamous hyperplasia of the nonglandular stomach in male and female mice, and with ulcers of the nonglandular stomach in female mice (NTP 1992b).

Epstein et al. (1970) treated infant Swiss albino mice with 0.11 to 110 mg PEG-20 Sorbitan Stearate in 0.9% saline at test volumes of 0.1 to 0.2 ml on days 1, 7, 14, and 21. The mice were injected subcutaneously (SC) in the nape of the neck, and were examined for signs of carcinogenicity daily during the first month, then monthly thereafter for the duration of the 49- to 53-week study. Mortality at weaning was 100% for the two highest doses, but only 2% in the low-dose group, compared to 14% to 19% in the vehicle and uninjected control groups. The only significant findings upon necropsy were solitary adenomas in 3 of 16 males and a lymphoma in 1 male (total dose = 6.6 mg PEG-20 Sorbitan Stearate) that survived to 49 weeks.

Shirai et al. (1982) investigated the effects of sodium chloride, PEG-20 Sorbitan Stearate, and N-ethyl-N′-nitro-N-nitrosoguanidine (ENNG) on gastric carcinogenesis in the male Wistar rat. The Polysorbate did not increase the incidence of tumors of the nonglandular stomach and glandular stomach when given subsequent to treatment with N-methyl-N′-nitro-N-nitrosoguanidine (MNNG), which was also the positive control chemical.

A concentration of 0.002% PEG-20 Sorbitan Stearate or PEG-20 Sorbitan Oleate inhibited metabolic cooperation (cell-cell communication) in Chinese hamster V79 cells in vitro (Trosko et al. 1981; 1982). This inhibition can result in tumor promotion via the deregulation of various complex cellular functions, such as proliferation, differentiation, immune response, and gene modulation. The tumor promoters appeared to inhibit contact inhibition or metabolic cooperation between cells, alter growth factor receptor sites, affect the immune system, and/or block chalones. Of the two Polysorbates, PEG-20 Sorbitan Stearate had an in vivo promoter response (Trosko et al. 1981).

PEG-20 Sorbitan Stearate at a dose of 6.6 mg did not promote the formation of tumors in neonatal ICR mice (Fujii 1991). The Polysorbate was injected into the necks of 49 neonatal mice within 24 hours of birth, and the mice were observed for 1 year. Four males (incidence = 16%) and four females (17%) had tumors: the males had lung tumors, two females (9%) had lung tumors, one female (4%) had lymphoma or leukemia, and one female (4%) had thyroid adenoma.

PEG-20 Sorbitan Stearate induced the cytoplasmic accumulation of transcripts of the proliferin gene family in mouse fibroblast C3H/10T1/2 cells (Parfett 1992). Proliferin protein is an antagonistic regulator of muscle-specific transcription, and can promote morphological transformation. The accumulation of proliferin transcripts occurred at or near the effective concentration for promotion of transformation.

The Polysorbates are not considered oral or dermal carcinogens, and were weak tumor promoters (cited in detail in Elder 1984). Skin tumors were produced by topical application of these compounds, but the observed tumors were mostly benign dermal tumors with a tendency toward regression. Various results were reported during studies in which the Polysorbates were injected subcutaneously. Investigators concluded that local sarcomas in the rat produced by long-continued repeated injections into the same subcutaneous site was not a valid index of chemical carcinogenicity. In a 21-week study, mixed cultures of epidermal and dermal cells from term fetuses of Balb/c mice were exposed to medium containing PEG-20 Sorbitan Oleate as a control. The Polysorbate did not produce the degree of in vivo malignancy or the same types of changes in morphology and cell differentiation as the test compound, 50 μg/ml of 7, 12-dimethylbenz[a]anthracene (DMBA) (Elder 1984).

Sorbitan Esters

Mice fed low concentrations of Sorbitan Stearate for 80 weeks had no difference in tumor type and incidence of tumors as compared to control animals. No carcinogenic effects were observed after undiluted Sorbitan Laurate was applied twice weekly to the clipped skin of the interscapular region of male Swiss mice for 73 weeks. Sorbitan Laurate was, however, a tumor promoter following induction with the tumor initiator DMBA. In a 75-week study, 5 of 50 mice developed a total of eight tumors (including one carcinoma), two of which regressed. Of the 100 control

mice, 1 had five tumors. In a 52-week study, daily application of 80 mg of Sorbitan Laurate (150 μg DMBA) resulted in 10 tumors in nine mice (treated once daily) and 33 tumors in 21 animals (twice daily). Undiluted Sorbitan Laurate was also active on mouse skin as a cocarcinogen with 0.003% to 0.3% DMBA. In the same study, Sorbitan Oleate and Sorbitan Trioleate were active as cocarcinogens on mouse skin when applied with 0.003% DMBA (Elder 1985).

Sorbitan Stearate was fed to 48 male and 48 female TO strain mice at dose levels of 0%, 0.5%, 20% or 40% of the diet for 80 weeks. Tumor type and incidence were two of the parameters studied. A majority of the tumors found in this study occurred either with comparable frequency in the test and control groups or more frequently in the control groups (Hendy et al. 1978).

Sorbitol

At a concentration of 18% in drinking water (3000–7000 mg/kg/day), hydrogenated starch hydrolysates (mixtures of polyhydric alcohols such as ~7.0% Sorbitol) were not carcinogens 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 any risk to humans exposed to normal concentrations of Sorbitol in the diet (Roe 1984).

Polyethylene Glycol

PEG-8 was not carcinogenic when administered orally to mice (30 weeks of dosing), intraperitoneally to rats (6 months of dosing), subcutaneously (20 weeks of dosing for rats; 1 year of dosing for mice), or when injected into the gastric antrum of guinea pigs over a period of 6 months (Andersen 1993).

COCARCINOGENICITY

Sorbitan Laurate was tested for both tumor promoting activity and carcinogenicity in the skin using 50 male Swiss mice. Sorbitan Laurate was applied to a 2 × 2-cm area of the interscapular region kept free of hair by periodic clipping. During the carcinogenicity experiment, Sorbitan Laurate was applied twice weekly to the skin for 73 weeks. All animals were checked twice weekly for skin lesions. No carcinogenic effect was detected, with 1 animal out of 50 developing one papilloma. Control groups of 240 male and female mice from the same colony were kept untreated and observed over their lifespan. One papilloma appeared and regressed in one control female and one skin papilloma and a carcinoma of skin appendages were each found in a control male. Additional control groups of 100 males and 100 females were observed for over 100 weeks and showed no signs of skin tumors. In the test of Sorbitan Laurate as a promoting agent, a single application of DMBA as a 1% solution in mineral oil was applied 1 week after the single application of the ester (dose not given), and, thereafter the ester was applied twice weekly for 75 weeks. Five of the 50 animals developed eight tumors, one of which regressed. One of the eight tumors was a carcinoma. Two nonconcomitant control groups received the DMBA and no further treatment. One of the 100 control mice developed five tumors (Saffiotti and Shubik 1963).

A study published by Setälä (1956) on the promoting and cocarcinogenic activity of a variety of nonionic-lipophilic-hydrophilic agents that included Sorbitan Laurate, Oleate and Trioleate. An initial single dose of 150 μg of DMBA (0.3% in paraffin) was painted on the backs of male mice (50 mice per group). The hair was cut from the treatment site twice weekly. The promoting agents were applied to the test site in doses that ranged between 51 to 87 mg once or twice daily, 6 days per week for 52 weeks. Animals receiving Sorbitan Laurate once or twice daily after initiation had 10 tumors in 9 animals and 33 tumors in 21 animals, respectively. The Sorbitan Oleate group had five tumors in four animals. No tumors were observed in animals that received Sorbitan Trioleate after initiation. Additional details are available in Table 9. Sorbitan Oleate and Trioleate were inactive as tumor promoters. Sorbitan Laurate was considered an active tumor promoter on mouse skin, apparently based on the finding that doubling the frequency of application increased significantly the mean incidence of tumor-bearing mice without increasing the dose of carcinogen.

Setälä (1956) also investigated the cocarcinogenic activity of Sorbitans Laurate, Oleate and Trioleate. DMBA of either 0.3% (150 μg), 0.03% (15 μg), or 0.003% (1.5 μg) was dissolved into the various Sorbitans and applied to the backs of mice (50 per group) three times per week. The hair was cut from the treatment site twice weekly. At the 0.3% DMBA dose the results were: Sorbitan Laurate, 240 tumors in 46 animals after 30 weeks; Sorbitan Oleate, 1 tumor in 1 animal after 10 weeks; Sorbitan Trioleate, 17 tumors in 8 animals after 17 weeks; and controls (DMBA in liquid paraffin), 200 tumors in 46 animals after 26 weeks. The

TABLE 9
Mean incidence of tumor-bearing mice during a 10-week period (Setälä 1956)

Compound tested for tumor-promoting capacity	Mean incidence of tumor-bearing mice (%)
PEG Sorbitan Stearate	63
PEG Sorbitan Palmitate	48
PEG Sorbitan Trioleate	37
PEG Sorbitan Oleate (Tween 80)	27
Sorbitan Laurate	2.9
Sorbitan Oleate	1.5
PEG Sorbitan Laurate	1.1
PEG Sorbitan Oleate (Tween 81)	0
Sorbitan Trioleate	0
PEG Sorbitol Tetraoleate	0

results for the 0.03% dose were: Sorbitan Laurate, 155 tumors in 31 animals after 30 weeks; Sorbitan Oleate, 168 tumors in 30 animals after 36 weeks; Sorbitan Trioleate, 130 tumors in 41 animals after 41 weeks; and controls (DMBA in liquid paraffin), 215 tumors in 39 animals after 34 weeks. At the 0.003% carcinogen dose, the results were: Sorbitan Laurate, 155 tumors in 35 animals after 52 weeks; Sorbitan Oleate, 25 tumors in 16 animals after 52 weeks; Sorbitan Trioleate, 57 tumors in 27 animals after 52 weeks; and controls (DMBA in liquid paraffin), 18 tumors in 13 animals after 52 weeks. Sorbitan Laurate and Sorbitan Trioleate were active on mouse skin as cocarcinogens when used as the solvent for 0.003% DMBA. Carcinomas did not develop on mouse skin when Sorbitan Oleate was used as a solvent for 0.003% DMBA.

TUMOR INHIBITION

Crispens and Sorenson (1990) tested combinations of $Cu(II)_2$ (3,5-diisopropylsalicylate)$_4$ (CuDIPS) a binuclear complex with immunostimulant and superoxide dismutase mimetic activities), PEG-20 Sorbitan Oleate, and cyclophosphamide for their effects on mortality and incidence of reticulum cell sarcoma in female SJL/J mice. Mice treated with CuDIPS and PEG-20 Sorbitan Oleate had a reduced survival rate and accelerated rate of tumorigenesis during the 52-week treatment period. PEG-20 Sorbitan Oleate plus cyclophosphamide caused a reduction in tumor incidence, as did the Polysorbate plus polyvinyl alcohol and other compounds. In general, high concentrations of PEG-4 and -20 Sorbitan Laurate, PEG-20 Sorbitan Palmitate, PEG-20 Sorbitan Stearate, and PEG-20 Sorbitan Oleate were cytotoxic to mouse Ehrlich ascites carcinoma cells, and produced reversible alterations in cellular membranes, inhibited respiration, and increased sensitivity to hyperthermia during in vitro studies. PEG-20 Sorbitan Laurate and PEG-20 Sorbitan Palmitate did not have in vivo tumor growth inhibition activities in mice with Ehrlich ascites carcinomas (Elder 1984).

Crispens and Sorenson (1991) reported that PEG-20 Sorbitan Laurate, PEG-20 Sorbitan Palmitate, and PEG-20 Sorbitan Stearate had little activity against reticulum cell sarcoma in SJL/J mice during a 52-week study, although PEG-20 Sorbitan Oleate had anticancer activity in the SJL/J tumor system. The apparent difference was attributed to the fatty acid components (saturated vs. unsaturated) of the Polysorbates.

When 50 Syrian hamsters were treated weekly for 25 weeks with benzo(a)pyrene via tracheal instillation, simultaneous administration of 5% PEG-20 Sorbitan Oleate reduced the number of benign and malignant neoplasms (Farrell 1974). These neoplasms included papillomas in the larynx and trachea, adenomas and adenocarcinoma in the bronchi, bronchioles, and lung periphery, and other neoplasms outside the respiratory tract. The vehicle was administered for 40 weeks, and from the 41st week to death, and the hamsters received PEG-20 Sorbitan Oleate, vehicle alone, or both. The total number of neoplasms observed after treatment with 3.4 mg benzo(a)pyrene in 0.2 ml of the 0.5% gelatin-in-saline vehicle was 24. Twelve neoplasms were observed after treatment with benzo(a)pyrene plus 5% PEG-20 Sorbitan Oleate. No malignant neoplasms were observed in the respiratory tract of hamsters given the gelatin-in-saline vehicle, PEG-20 Sorbitan Oleate, or both; hamsters given the Polysorbate plus carcinogen only in the first instillation also had no malignant neoplasms. Hyperplasia of type II alveolar epithelial cells was observed via electronmicroscopy in animals without neoplasms.

In a study using 10 male CDF_1 mice per group, the simultaneous IP administration of 5000 mg/kg PEG-20 Sorbitan Oleate increased the chemotherapeutic activity of Adriamycin against murine leukemia P388 (Harrison, Cusic, and McAfee 1981). This action was due to an apparent reduction of plasma volume. In another study (Stavrovskaya et al. 1975), PEG-20 Sorbitan Oleate at concentrations greater than 0.1% increased the sensitivity of colcemid-resistant transformed mouse cells in vitro.

In an earlier study, Casazza et al. (1978) reported that Adriamycin in a 10% aqueous solution of PEG-20 Sorbitan Oleate produced significant increases of antitumor activity in mice against ascites tumors (L 1210 leukemia), disseminated leukemias (transplanted leukemias originally induced by Gross and Moloney leukemia viruses), and solid tumors (sarcoma 180 and MS-2 sarcoma) when the solution was administered intravenously. Toxicity of the antitumor drug, however, was enhanced. When the solution was administered intraperitoneally, no increase in antitumor activity was observed and toxicity was increased significantly.

Menon et al. (1984) investigated the effects of a PEG-20 Sorbitan Oleate vehicle on the natural resistance of sarcoma-180 tumor to the protein synthesis inhibitor, bouvardin. In this study, 10% PEG-20 Sorbitan Oleate enhanced cytotoxicity and increased the life span of Swiss mice bearing the sarcoma. In vitro, sarcoma cells exposed to bouvardin alone had inhibition of uridine incorporation by 46%, whereas bouvardin plus the Polysorbate inhibited uridine incorporation by 66%.

In other studies, PEG-20 Sorbitan Oleate at a concentration of 10% enhanced antitumor activity of adriamycin against murine P388 leukemia (Chitnis, Menon, and Gude 1984). At concentrations of 5% to 10% (0.1–0.2 ml/mouse, IP), the Polysorbate had anticancer activity against reticulum cell sarcoma in female SJL/J mice (Crispens, Porter, and Sorenson 1986; Crispens and Sorenson 1988). Kubis et al. (1979) reported that 5% (500 mg/kg, IP) PEG-20 Sorbitan Oleate had a cytotoxic effect on Ehrlich ascites tumors in Balb/c mice.

CLINICAL ASSESSMENT OF SAFETY

Oral Toxicity

Polysorbates are commonly used emulsifiers in foods, and are approved by the FDA as direct and indirect food additives for human consumption (see Noncosmetic Use). The typical concentration of use of Polysorbates as food additives is 0.4% (HSDB 1996).

PEG-20 Sorbitan Stearate and PEG-20 Sorbitan Oleate have a toxicity rating of practically nontoxic (1/6), with a probable oral lethal dose in humans >15 g/kg.

Polysorbates have been used in the treatment of lipid malabsorption syndromes. PEG-20 Sorbitan Oleate was described as useful for promoting fat absorption from the alimentary tract and no harmful effects were reported. It was judged harmless for human consumption in amounts of at least 6.0 g/day. A 4-month-old male infant (<8 lbs) consumed 19.2 g PEG-20 Sorbitan Oleate daily for 2 consecutive days with no other food; the infant had loose stools, but no evidence of toxicity. When 13 premature and two full-term infants with steatorrhea were given four 200-mg daily doses of PEG-20 Sorbitan Laurate, the infants had no increase in fat absorption, but had no adverse effects with respect to anorexia, vomiting, defecation, or growth. In another study, nine premature infants were given 0.179 to 0.335 g/kg PEG-20 Sorbitan Oleate as a dietary supplement for a period of 4 consecutive days. The investigators did not report any adverse effects. Adults fed 20 g PEG-20 Sorbitan Stearate as a single dose had no changes in gastric motility or acidity, and no adverse effects were observed (Elder 1984).

Sorbitan Esters

Three clinical assessments have evaluated the oral toxicity of Sorbitan Stearate. One dose of 20 g was administered to five subjects, two of whom had increased gastric motility. One subject had an increase in free gastric acidity, and all subjects had normal gastric juices. In another study, nine patients were given 3 g Sorbitan Stearate twice daily for 28 days. Seven patients had normal gas patterns, one had more, and one had less at the end of the observation period. Seven patients had no change in gall bladder function, the eighth had increased emptying time, and the ninth patient had fainter visualization. Normal radiographic intestinal patterns were observed for all nine patients. In the third study, 42 subjects ingested 6 g Sorbitan Stearate daily for 28 days. Eleven subjects had albumin in their urine at the end of the study, and four had glycosuria; one of the four, however, was diabetic, and another had an abnormal glucose tolerance test. No significant changes were found in hemoglobin content, hematocrit, red cell count, or red cell fragility, and blood chemistry values were normal except in one patient who had slightly elevated total serum bilirubin (Elder 1985).

Parenteral Toxicity

From 1983 to 1984, low-birth-weight infants in neonatal intensive care units were given 25 U/ml supplements of vitamin E (DL-α-tocopheryl acetate) solubilized in 9% PEG-20 Sorbitan Oleate and 1% PEG-20 Sorbitan Laurate. The supplements were administered intravenously as 1 to 2-ml vials, usually admixed with a parenteral nutrition solution. Before the vitamin solution was withdrawn from the market, 38 premature infants died and 43 more became seriously ill. In one study, 14 of 17 affected infants that had been treated with the vitamin supplement died. Affected infants had dose-related, progressive deterioration characterized by unexplained thrombocytopenia, renal dysfunction, azotemia, hepatomegaly, cholestasis, ascites, hypotension, and metabolic acidosis. Oxalic acid-type crystals were observed in the distal renal tubules and collecting ducts. These effects were attributed to the high concentration of Polysorbate emulsifiers in the vitamin E supplement (Bove et al. 1985; Lorch et al. 1985; McKean, Peske, and Koo 1987; Pesce and McKean 1989; McKean 1992; Smolinske 1992). At microscopic examination of autopsy samples, lesions indicative of progressive injury were observed, including Kupffer cell exfoliation, hepatocytolysis, sinusoidal dilatation with accumulation of cellular debris and "free-floating" cells (<1 week after infusion), attenuation of liver cell plates with extreme panlobular-congestion (1–2 weeks), cholestasis, early intralobular fibrosis (2–3 weeks), and ultimately, marked fibrosis with sinusoidal "obliteration." Regression was not apparent after discontinuation of the parenteral solution (Balistreri et al. 1985).

Tissue extracts from the neonates had unmetabolized Polysorbates at concentrations of up to 200 μg/ml (McKean 1992). An infant that died had 100 μg/ml of Polysorbates in ascites fluid (McKean and Pesce 1985). Compared to the membranes of human adults, human infant membranes were more sensitive to the effects of the surfactants and did not efficiently metabolize Polysorbates (McKean 1992). In adults, 90% of administered Polysorbates was eliminated via the urine in 24 hours. In infants, only the polyoxyethylated metabolite (not identified) was excreted (Pesce and McKean 1989).

Alade et al. (1986) reported that the mean in vitro response of human lymphocytes to phytohemagglutinin (PHA) was stimulated by vitamin E (α-tocopherol acetate), whereas the commercial supplement and PEG-20 Sorbitan Oleate inhibited the PHA response by 37% and 44%, respectively. The percentage of T11 cells was also decreased by addition of the supplement and either or both of the Polysorbates. Based upon animal toxicology data on PEG-20 Sorbitan Oleate, the investigators suggested that the supplement's toxicity was due to the catabolism of the Polysorbate vehicle. Oleic acid and polyoxyethylene moieties released during in vivo hydrolysis of PEG-20 Sorbitan Oleate could have contributed to the pulmonary deterioration and renal failure of the affected infants. In addition, the renal failure and azotemia could have been due to the conversion of the other constituent, ethylene oxide, to ethylene glycol, a known nephrotoxic agent.

Skin Irritation and Sensitization

PEGs Sorbitan/Sorbitol Fatty Acid Esters

One case report described contact sensitivity (on the forehead) to PEG-40 Sorbitan Lanolate in a 28-year-old male using a styling gel (Pazzaglia et al. 1995). Volunteers were patch tested with PEG-40 Sorbitan Lanolate (50 subjects), PEG-50 Sorbitol Hexaoleate (200 subjects), PEG-40 Sorbitan Peroleate (208 subjects), and PEG-30 Sorbitol Tetraoleate Laurate (50 subjects). The test materials (concentration not specified) were each applied to a 1 × 1-inch square of absorbent cotton twill, which was sealed onto the skin with a 2 × 2-inch elastic adhesive patch. The patches were removed after 72 hours, and the skin sites were observed for irritation. This procedure was repeated at the

same site 7 days after the removal of the patch. Under the conditions of this study, PEG-40 Sorbitan Lanolate, PEG-50 Sorbitol Hexaoleate, PEG-40 Sorbitan Peroleate, and PEG-30 Sorbitol Tetraoleate Laurate were nonsensitizing (CTFA 1998b).

Polysorbates

When a number of emulsifiers, including a mixture of 5% PEG-20 Sorbitan Stearate and 5% PEG-20 Sorbitan Oleate in petrolatum, was tested for contact sensitization (Hannuksela, Kousa, and Pirila 1976), two patients had allergic reactions (0.2%) and two had "toxic reactions" (0.2%). For this study, epicutaneous tests were performed with the Finn chamber method using 1206 patients with eczema. The occlusive patches were in place for 24 hours, and the sites were evaluated 20 minutes after removal, and after 2, 4, and 5 days after application. Irritant reactions were marked on the first or second day, and disappeared or became more faint within 4 to 5 days. One patient who reacted to the Polysorbates also reacted to polyoxyethylene oxypropylene stearate and PEG-n Sorbitol Lanolin. The second patient had reactions to a polyoxyethylene sorbitol lanolin derivative, balsam of Peru, neomycin, bacitracin, and tetramethylthioram disulphide (TMTD).

In a similar study, 10% PEG-20 Sorbitan Palmitate in petrolatum did not produce sensitization in 47 patients with chronic or recurrent (>1 year) inflammatory skin diseases (Pasche-Koo et al. 1994). The same concentration of PEG-20 Sorbitan Oleate caused sensitization in one patient with eczema, but no sensitization was observed in other patients. The control group for this study consisted of 10 healthy volunteers.

A group of 737 patients with suspected cosmetic-related contact dermatitis were patch-tested with six emulsifiers (Tosti et al. 1990). PEG-20 Sorbitan Palmitate and PEG-20 Sorbitan Oleate were tested at 10% in petrolatum. Thirty-nine patients (5.3%) had one or more positive patch sites to the emulsifiers. Of that number, two patients reacted to PEG-20 Sorbitan Palmitate, one reacted to PEG-20 Sorbitan Oleate, and three reacted to both Polysorbates. The typical reaction sites were the hands and face. The sensitization was classified as clinically relevant in four patients; three had been sensitized by leave-on cosmetics and one by an antimycotic cream containing 0.1% PEG-20 Sorbitan Oleate, 1.5% PEG-20 Sorbitan Stearate, 2% sorbitan stearate, and other ingredients.

During a 4-hour patch test using the Hill Top chamber, undiluted PEG-20 Sorbitan Oleate caused positive reactions in only 1 of 27 patients (incidence = 4%). The comparable reactivity of 20% sodium dodecyl sulfate was eight of 27 patients (30%). As a result, PEG-20 Sorbitan Oleate was not classified as a human skin irritant (York et al. 1996).

PEG-20 Sorbitan Oleate at a concentration of 100% was noncorrosive to the skin of humans during an in vitro assay (Perkins, Osborne, and Johnson 1996). The test substance was applied topically to the stratum comeum of human skin cultures and cytotoxicity was measured as decreased MTT metabolism (see General Biology—Cytotoxic Effects). In a study by Gay et al. (1992), PEG-20 Sorbitan Oleate was not irritating to a living skin equivalent. This in vitro system was comprised of an organotypic coculture of human dermal fibroblasts in a collagen-containing matrix overlaid with human keratinocytes that have formed a stratified epidermis. Irritation was determined by the cytotoxicity values of the MTT assay. The results correlated with those reported in in vitro studies using rabbit and human skin.

Roguet et al. (1994) reported that PEG-20 Sorbitan Laurate was nontoxic to Episkin reconstituted human epidermis. The LC_{50} of PEG-20 Sorbitan Laurate in human keratinocyte monolayers (MTT assay) was 1.22 mg/ml.

The Polysorbates did not cause dermal irritation or sensitization in patch tests that used up to 50 subjects exposed to concentrations as high as 100%. When product formulations containing 2% to 8.4% PEG-20 Sorbitan Laurate were tested for 24-hour primary irritation, minimal to mild irritation was observed. A bubble bath (0.03%–6% PEG-20 Sorbitan Laurate) was moderately to severely irritating to the skin during cumulative irritancy tests. Lotions and creams containing 4.0% PEG-20 Sorbitan Palmitate were slightly irritating in a cumulative irritancy test. In similar tests, a cream containing 6.0% PEG-20 Sorbitan Stearate was essentially nonirritating, and a cream containing 1% PEG-20 Sorbitan Trioleate was slightly irritating to the skin. PEG-20 Sorbitan Laurate in formulation at concentrations of 0.3%–2.4% was nonsensitizing and nonphotosensitizing in Schwartz-Peck prophetic patch tests using up to 197 subjects. Irritation and sensitization were not observed when a shaving preparation containing 0.6% PEG-20 Sorbitan Stearate was evaluated in a similar study using 197 subjects. Mild irritation was observed when makeup containing 0.6% PEG-20 Sorbitan Oleate was tested using 303 subjects. No signs of sensitization, but minimal to mild irritation were observed when shaving foams and a moisturizer containing 2.5% PEG-20 Sorbitan Stearate, 2.5% PEG-20 Sorbitan Oleate, or 1.0% PEG-20 Sorbitan Trioleate were evaluated during a 48-hour prophetic patch and in-use test using up to 204 subjects. In other studies, formulations containing up to 6.0% of the Polysorbates were minimally irritating but not sensitizing (Elder 1984).

The cutaneous toxicities of PEG-20 Sorbitan Laurate and two other surfactants (sodium dodecyl sulfate [SDS] and Triton X-100) were determined using cultured human keratinocytes (Shivji et al. 1994). Three end points were evaluated: cytotoxicity as determined by crystal violet staining (CVS), the release of [^{3}H]arachidonic acid, and the regulation of the proinflammatory cytokine interleukin-1α (IL-1α) message in keratinocytes. Arachidonic acid was released from membrane phospholipids after induction by dermal irritants. IL-1α was constitutively produced and stored by keratinocytes and was released upon injury to the cells, thereby stimulating the release of other cytokines. The IL-1α message was semiquantitated using the reverse transcription polymerase chain reaction. PEG-20 Sorbitan Laurate was the least toxic of the surfactants tested. PEG-20 Sorbitan Laurate at concentrations of 0.55% and 0.04% reduced cell viability by 50% (CVS_{50}) after 1 and 24 hours

of treatment, respectively. These values were obtained from dose-response curves. PEG-20 Sorbitan Laurate had a time-dependent toxic effect, but not as prominent as that observed after treatment with SDS or Triton X-100. PEG-20 Sorbitan Laurate required greater exposure time to exert an effect on the treated keratinocytes.

The concentration of PEG-20 Sorbitan Laurate needed to release 50% of the arachidonic acid (AAR_{50}) in normal keratinocytes was 0.20% after 2 hours of treatment. The highest concentration of PEG-20 Sorbitan Laurate tested, 0.01%, caused an induction of IL-1α messages. PEG-20 Sorbitan Laurate upregulated the expression of IL-1α mRNA compared to the vehicle control (Shivji et al. 1994).

Sorbitan Esters

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, when 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).

The Sorbitan Esters were minimal to mild skin irritants in humans. Products containing low concentrations of Sorbitan Stearate were mild irritants in 21-day cumulative irritation studies. A Schwartz Prophetic Patch test using 30% Sorbitan Laurate in water or undiluted Sorbitan Laurate did not produce signs of irritation in 10 or 50 subjects, respectively. In two Schwartz Prophetic Patch tests (60 subjects total), high concentrations of Sorbitan Sesquioleate produced no reactions. Several products containing 1.75% to 2% Sorbitan Oleate have been tested on human subjects. In four 21-day cumulative irritation studies, the products were mildly irritating; the specific ingredient(s) causing irritation was not determined. No irritation was observed in maximization tests using Sorbitan Oleate. Two of 53 subjects had mild irritation during a product usage study of Sorbitan Oleate. Sorbitan Tristearate, in a Schwartz Prophetic Patch test, produced no irritation in 211 panelists. Sorbitan Palmitate–containing skin formulations were slightly irritating to humans in a 21-day cumulative irritancy test using 34 subjects. Products containing 5% Sorbitan Trioleate were slightly irritating in 21-day cumulative irritancy tests, a Shelanski-Jordan repeat-insult patch test (RIPT), modified Schwartz-Peck predictive patch tests, and in a 4-week usage test. Results from three RIPTs (involving a total of 420 subjects) indicated that Sorbitan Stearate was not a sensitizer. Four RIPTs involving 339 panelists classified Sorbitan Oleate-containing products as nonsensitizers. In a Shelanski-Jordan RIPT (206 subjects), a skin care product containing Sorbitan Palmitate was neither an irritant nor a sensitizer. Human tests for sensitivity to Sorbitan Sesquioleate indicated that the compound was a nonsensitizer. In five RIPTs involving 352 subjects, results indicated that none of the five products containing 1% to 3% Sorbitan Sesquioleate was a sensitizer; some subjects, however, experienced mild irritation. Products containing up to 2% of either Sorbitan Stearate or Sorbitan Oleate were nonphototoxic and nonphotoallergenic (Elder 1985).

Polyethylene Glycol

In clinical studies, PEG-6 and PEG-8 induced mild sensitization in 9% and 4% of 23 male subjects tested, respectively. However, later production lots of PEG-6, as well as PEG-75, did not cause reactions in any of the 100 male and 100 female subjects tested. A product formulation containing 3% PEG-8 induced minimal to mild irritation (induction phase) in over 75% of 90 volunteers participating in a skin irritation and sensitization study. Responses (not classified) were noted in 22 subjects at the 24-hour challenge reading. Cases of systemic toxicity and contact dermatitis in burn patients were attributed to PEG-based topical ointments. The ointment that induced systemic toxicity contained 63% PEG-6, 5% PEG-20, and 32% PEG-75 (Andersen 1993).

Ocular Irritation

In clinical ocular irritation tests, PEG-20 Sorbitan Laurate was nonirritating (Elder 1984).

PEG-20 Sorbitan Laurate markedly increased the permeability of the corneal epithelium in the human eye to fluorescein. The test compound was instilled at a concentration of 1% in saline (0.9% mixed with 5% Tris buffer, pH = 7.4; fluorescein added for final concentration of 0.75%). Concentrations of PEG-20 Sorbitan Laurate up to 40% did not produce adverse ocular effects in the volunteers tested (Marsh and Maurice 1971).

Enucleated human eyeballs was used in an ocular toxicity assay as a model for human corneal damage after exposure to various chemicals, including PEG-20 Sorbitan Laurate. The majority of human eyeballs were enucleated 3 to 24 hours after death. All were stored at 4°C for at least 12 hours before the experiment, which took place 24 to 72 hours after death. The isolated eyeballs were kept in a temperature-control chamber (32–36°C), held vertically in clamps, and irrigated with Hanks' balanced salt solution at the upper limbus. The eyeballs were examined using a Haag-Streit Slit Lamp before and after treatment, and the corneal thickness was measured. After 90 minutes, 20- and 100-μl volumes of PEG-20 Sorbitan Laurate were applied at the superior limbus for 10 seconds and 1 minute, respectively. The larger volume was applied in 20-μl aliquots at 10-second intervals. The eyeballs were rinsed for 10 seconds with saline. Cornea thickness was measured every 30 minutes; the total time of the experiment was 7 to 8 hours. Exposure to PEG-20 Sorbitan Laurate had little effect on corneal deturgescence in isolated human eyeballs, compared to controls. Increased granularity of the epithelium and fluorescein staining in the contact area were observed after treatment with PEG-20 Sorbitan Laurate (Berry and Easty 1993).

SUMMARY

The PEGs Sorbitan/Sorbitol Fatty Acid Esters are ethoxylated sorbitan and sorbitol esters of fatty acids that function as surfactants in cosmetic formulations. These ingredients were

used in a total of 81 cosmetic formulations in 1998. The Polysorbates, which are food additives, were used in 1418 formulations. They are formed by the esterification of sorbitol or sorbitan with a fatty acid, followed by the chemical addition of ethylene oxide. Typical impurities can include the free fatty acids, alcohol, peroxides, isosorbide ethoxylates, and other compounds; 1,4-dioxane and other water-soluble by-products are removed during the manufacturing process.

Few data on the ingredients in this review were available; therefore, relevant data from the previous CIR safety assessments on the Polysorbates (other PEGs Sorbitan Fatty Acid Ester), PEGs, and Sorbitan Esters were included in this report as a further basis for assessing their safety in cosmetics.

During feeding studies, the Polysorbates were absorbed and hydrolyzed by blood and pancreatic lipases. The fatty acid moiety was absorbed and metabolized as any other dietary fatty acid, and the PEG Sorbitan moiety was excreted mainly in the urine. The gastrointestinal absorption of PEGs was dependent on the molecular weight; the greater the molecular weight, the lesser the absorption that occurs. In oral and IV studies, the PEGs were not metabolized and were rapidly eliminated in the feces and urine. PEGs were readily absorbed through damaged skin.

A number of cytotoxicity assays has been performed on the Polysorbates; they caused both membrane damage and reduced mitochondrial activity. A concentration of 5% PEG-20 Sorbitan Oleate in rats caused the "destruction" of the mitochondria of the epithelium of the small intestine of Wistar rats. The Polysorbate (concentration = 10%) caused a portion of the microvilli to disappear with flattening of the surfaces of the epithelial cells. PEG-20 Sorbitan Oleate had immunosuppressive effects in Balb/c mice that had been immunized with ovalbumin. PEG-20 Sorbitan Oleate was also a histamine-releasing agent, and increased recruitment of peritoneal macrophages without modifying phagocytic activity. PEG-20 Sorbitan Oleate (100 mg/ml) depressed cardiac potential in dogs and guinea pigs; the Polysorbate reduced mean arterial blood pressure and left ventricular dP/dt.

The Polysorbates had low toxicity in both acute and long-term toxicity studies using animals. In rats, the LD_{50} values for these ingredients were >5 to >38.9 g/kg (oral), ~1.4 g/kg (IV), and 0.7 to >5 ml/kg (IP). When administered to rats by IP injection, 16% PEG-20 Sorbitan Laurate and 32% PEG-20 Sorbitan Oleate decreased locomotor activity. During an inhalation toxicity study, PEG-20 Sorbitan Oleate (7%; 0.1 to 0.2 ml) was relatively nontoxic. The Sorbitan Esters and PEGs also were relatively nontoxic to animals.

During a 14-day feeding study of 3000 to 50,000 ppm PEG-20 Sorbitan Oleate, the high dose caused decreased body weight in male rats and mice, but no other clinical findings were reported. A vehicle containing 9% PEG-20 Sorbitan Oleate and 1% PEG-20 Sorbitan Laurate was mildly hepatotoxic to rabbits and, when given intraperitoneally, caused massive peritoneal fibrosis and degeneration of the kidneys in mice and rats. No adverse effects were observed in chicks fed 2% to 5% PEG-20 Sorbitan Stearate for 7 weeks. Rats fed 10% of the Polysorbate for 8 weeks had diarrhea for the first few days of treatment, but no other signs of toxicity. Rats fed 1.5 ml PEG-20 Sorbitan Oleate (1%–4%) for 3 months had congestive and degenerative changes in the heart, liver, and kidneys. In 6-week studies using rats and monkeys, PEG-4 Sorbitan Stearate, PEG-20 Sorbitan Stearate, and PEG-5 Sorbitan Oleate produced no significant adverse effects. In dermal toxicity studies, the PEGs did not cause signs of toxicity other than transient, mild erythema. Evidence of systemic toxicity was only observed in rabbits that received repeated topical applications of a PEG-based cream to abraded skin. Rats fed 1% to 4% Sorbitan Laurate for 6 weeks had decreased growth rates, and hamsters fed 15% for 68 days had degenerative changes of the gastrointestinal tract, and other lesions. Similar changes were observed in rats fed 25% Sorbitan Laurate for 70 days. Rhesus monkeys fed 2 g/day had no signs of toxicity after 6 weeks of treatment.

Growth retardation and diarrhea were noted in subchronic feeding studies of up to 10% PEG-20 Sorbitan Stearate using mice. Diarrhea in these and other studies was attributed to the high concentrations of the unabsorbed PEG Sorbitan moiety in the intestinal lumen. PEG-20 Sorbitan Oleate (up to 50,000 ppm) was nontoxic to rats and mice during a 13-week feed study. A concentration of 25% PEG-20 Sorbitan Laurate caused microscopic changes of the urinary bladder, spleen, kidneys, and gastrointestinal tract in rats during a 21-week study. The PEGs were nontoxic during a 90-day oral toxicity study using rats. Feeding of 10% to 25% Sorbitan Laurate for 90 days to 23 weeks caused decreased body and organ weights, diarrhea, and hepatic lesions in rats.

During a chronic toxicity study using hamsters, 5% to 15% PEG-20 Sorbitan Laurate caused microscopic lesions of the urinary bladder, kidneys, spleen, and gastrointestinal tract. In monkeys, 1 g/day PEG-20 Sorbitan Laurate did not cause adverse effects after 17 months of treatment. Rats fed up to 2% PEG-20 Sorbitan Laurate for over 2 years had no signs of toxicity. PEG-20 Sorbitan Stearate, PEG-20 Sorbitan Oleate, and PEG-20 Sorbitan Tristearate at concentrations <20% were nontoxic in long-term feeding studies using mice, rats, dogs, and hamsters. At concentrations of 20%, these Polysorbates caused some growth retardation and diarrhea, and had minor effects on longevity and reproduction. Studies using 2% PEG-20 Sorbitan Palmitate and PEG-20 Sorbitan Trioleate were also negative. In chronic studies, dogs fed 2% PEG-8, PEG-32, or PEG-75 for 1 year had no adverse effects; rats fed 5% Sorbitan Laurate for 2 years had no signs of toxicity, but only 15% of the treated and control rats survived to the end of the study.

The Polysorbates were nonirritating to mildly irritating in both in vivo and in vitro ocular irritation assays. The concentrations tested ranged from 1% to 100%. PEG-6 and PEG-75 did not cause corneal injuries when instilled into the conjunctival sac of rabbits, but 35% PEG-8 and 0.1 ml PEG-32 (melted in water bath) induced mild ocular irritation. Sorbitan

Laurate (30%–100%) was not an ocular irritant in Draize ocular irritation tests using rabbits.

The Polysorbates had little potential for rabbit and mouse skin irritation in acute studies. Moderate to strong sensitization to PEG-20 Sorbitan Laurate was observed in a Magnusson-Kligman guinea pig maximization test; PEG-20 Sorbitan Oleate and PEG-20 Tristearate were not sensitizers. PEG-20 Sorbitan Laurate (1%) did not have comedogenic potential in rabbits. The Sorbitan Esters were generally mild skin irritants, but did not cause sensitization in animals. The PEGs were neither irritants nor sensitizers.

In teratology studies of thalidomide, the PEG-20 Sorbitan Laurate vehicle (10 ml/kg) had no effect on the developing mouse embryo. In other studies, reproductive and developmental effects were seen primarily at exposure levels that were maternally toxic. PEG-20 Sorbitan Laurate caused dose-dependent malformations of offspring when administered to Swiss and NMRI mice via IP injections. In the Chernoff-Kavlock assay using Alpk/AP rats, 10 ml/kg/day PEG-20 Sorbitan Laurate reduced offspring litter size, survival, and weight gain when the Polysorbate was administered intraperitoneally, but the parameters did not differ from controls after dermal, oral, or subcutaneous administration. In another study using rats, PEG-20 Sorbitan Laurate had a maternal no-observable-effect level (NOEL) of 500 mg/kg/day, a maternal low effect level of 5000 mg/kg/day, and a developmental NOEL of >5000 mg/kg/day.

PEG-20 Sorbitan Laurate, PEG-20 Sorbitan Palmitate, PEG-20 Sorbitan Stearate, and PEG-20 Sorbitan Oleate caused serious developmental effects in sea urchin embryos when administered at concentrations as low as 0.004% in sea water. Mice fed 10% PEG-20 Sorbitan Stearate or PEG-20 Sorbitan Laurate during a multigeneration study had offspring with decreased weanling weights, significantly smaller litters, and delivered more dead fetuses than mice of the control group. PEG-20 Sorbitan Oleate was not teratogenic in a rat whole-embryo culture study. In in vivo studies using neonatal rats, PEG-20 Sorbitan Oleate (1%–10%, IP injection) accelerated maturation, prolonged the estrous cycle, and induced chronic estrogenic stimulation. The ovaries were without corpora lutea and had degenerative follicles, and the uterus had epithelial squamous cell metaplasia and cytological changes. PEG-20 Sorbitan Oleate (2500 mg/kg/day in one study; 1.25 ml/l drinking water in another) and PEG-20 Sorbitan Stearate (0.1%–10% in one study; 5200 mg/kg/day in another) did not cause developmental effects in rats and mice, but PEG-20 Sorbitan Oleate in drinking water increased locomotor activity and exploratory behavior of offspring of treated rats.

The PEG monomer, ethylene glycol, and certain of its monoalkyl ethers are reproductive and developmental toxins. The CIR Expert Panel concluded that, as the PEGs Sorbitan and Sorbitol Esters are chemically different from the alkyl ethers of ethylene glycol and the alkyl ethers are not present as impurities, these ingredients pose no reproductive or developmental hazard. In subchronic and chronic oral toxicity studies, the PEGs did not cause adverse reproductive effects.

The Polysorbates were nonmutagenic in a number of bacterial and mammalian systems, with the exception of PEG-20 Sorbitan Stearate, which produced both positive and negative results in genotoxicity assays.

In carcinogenicity studies, feeding of PEG-20 Sorbitan Oleate (up to 50,000 ppm) to rats and mice resulted in equivocal evidence of carcinogenicity; the male rats had an increased incidence of pheochromocytomas. The test compound was associated with inflammation and squamous hyperplasia of the nonglandular stomach in mice and with ulcers of the nonglandular stomach in female mice. PEG-20 Sorbitan Stearate did not increase the incidence of neoplasms in the nonglandular stomach and glandular stomach when administered with the carcinogens ENNG and MNNG. In general, the Polysorbates were not oral or dermal carcinogens, and were weak tumor promotors. PEG-20 Sorbitan Stearate and PEG-20 Sorbitan Oleate (0.002%) inhibited metabolic cooperation in V79 Chinese Hamster cells in vitro, which could result in tumor promotion. PEG-20 Sorbitan Stearate has been reported to have an in vivo promoter response, and the Polysorbate induced the cytoplasmic accumulation of proliferin transcripts in mouse fibroblasts; proliferin is an antagonistic regulator of muscle-specific transcription, and can promote morphological transformation. The Polysorbates also had antitumor activity in animal studies. PEG-8 was noncarcinogenic in studies using mice, rats, and guinea pigs. Sorbitan Laurate and Sorbitan Stearate were also noncarcinogenic. At concentrations ≥10%, Sorbitan Laurate was a tumor promoter in mouse skin.

The Polysorbates were nontoxic by the oral route in clinical studies, but a Polysorbate vehicle (9% PEG-20 Sorbitan Oleate, 1% PEG-20 Sorbitan Laurate) for a neonatal parenteral supplement caused the deaths of 38 premature infants. The symptoms and lesions observed included pulmonary deterioration, hepatomegaly, metabolic acidosis, and renal failure. Investigators concluded that human infant membranes were more sensitive to the effects of the Polysorbates and could not efficiently metabolize the compounds. Oleic acid and PEG moieties released during in vivo hydrolysis of PEG-20 Sorbitan Oleate could have contributed to the pulmonary deterioration and renal failure, as could ethylene glycol formed from ethylene oxide moieties.

The Polysorbates had little potential for human skin irritation, sensitization, and phototoxicity in extensive clinical studies. PEG-20 Sorbitan Oleate at a concentration of 100% was noncorrosive, and it and PEG-20 Sorbitan Laurate were not irritating to living skin equivalents. The PEGs were nonsensitizers, but cases of systemic toxicity and contact dermatitis were observed in burn patients that were treated with PEG-based topical ointments. The Sorbitan Esters had the potential to cause cutaneous irritation in humans, and could cause sensitization in patients with damaged skin. Sorbitan Stearate and Sorbitan Oleate were not photosensitizing; Sorbitan Laurate, Sorbitan Palmitate, Sorbitan Sesquioleate, and Sorbitan Trioleate did not absorb UVA or UVB light, suggesting that these compounds were not photosensitizers.

In clinical ocular irritation studies, PEG-20 Sorbitan Laurate was nonirritating, but at a concentration of 1%, it markedly increased the permeability of the corneal epithelium to fluorescein in the human eye. PEG-20 Sorbitan Oleate was classified as an ocular irritant, but further details were not available.

DISCUSSION

The CIR Expert Panel has reviewed previously the safety of the Polysorbates, which are specific PEGs Sorbitan/Sorbitol Fatty Acid Esters, as well as that of their components (sorbitan esters, fatty acids, and PEGs). The larger-molecular-weight PEGs Sorbitan Fatty Acid Esters and their components are known not to be toxic. It is generally recognized that the PEG monomer, ethylene glycol, and certain of its monoalkyl ethers are reproductive and developmental toxins. Given the methods of manufacture of the PEGs Sorbitan/Sorbitol Fatty Acid Esters, there is no likelihood of ethylene glycol or its alkyl ethers being present, and the ingredients are chemically different from the ethylene glycol alkyl ethers of concern.

The Expert Panel was concerned about the lack of data on dermal absorption and/or reproductive and developmental toxicity of the smaller-molecular-weight ingredients. For example, the molecular weights of PEG-2 Sorbitan Isostearate, PEG-3 Sorbitan Stearate, and PEG-4 Sorbitan Laurate are approximately 518 to 562 Da. In contrast, the molecular weights of PEG-20 Sorbitan Stearate and PEG-20 Sorbitan Laurate are approximately 1228 to 1310 Da. Given that the smallest ingredients of this family have relatively large molecular weights, the Panel expressed the view that the penetration into the skin of the smaller-molecular-weight polymers would not be great. This, coupled with the available data on the components of the PEGs Sorbitan/Sorbitol Fatty Acid Esters, led to a basic conclusion of safety.

The Expert Panel's "safe for use" conclusion is based on historical data on the concentration of use of certain of these ingredients. Accordingly, the "present practices of use" in the conclusion means that the Expert Panel does not expect uses of PEGs Sorbitan Fatty Acid Esters, including those not currently used, to exceed 25%. Although there were single-insult patch test data showing these ingredients were not sensitizers at certain concentrations, the Expert Panel did not establish a maximum use concentration on that basis because such patch testing was considered inappropriate to establish a level above which the ingredient would be considered a sensitizer.

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. The Expert Panel recommends that cosmetic formulations containing these PEGs not be used on damaged skin.

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 recognized that several of the Polysorbates enhanced skin penetration of other chemicals, and recommended that care should be exercised in using these ingredients in cosmetic products where the penetration of other ingredients is a concern.

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 authors' hypothesis that this effect may be a mechanism in cocarcinogenesis, but concluded that this was only an hypothesis and does not demonstrate a link between DNA repair inhibition and 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 is not likely to be relevant to the use of these PEG Sorbitan/Sorbitol fatty acid esters in cosmetic formulations.

CONCLUSION

The CIR Expert Panel concludes that PEG-20 Sorbitan Cocoate; PEG-40 Sorbitan Diisostearate; PEG-2, -5, and -20 Sorbitan Isostearate; PEG-40 and -75 Sorbitan Lanolate; PEG-10, -40, -44, -75, and -80 Sorbitan Laurate; PEG-3 and -6 Sorbitan Oleate; PEG-80 Sorbitan Palmitate; PEG-40 Sorbitan Perisostearate; PEG-40 Sorbitan Peroleate; PEG-3, -6, -40, and -60 Sorbitan Stearate; PEG-20, -30, -40, and -60 Sorbitan Tetraoleate; PEG-60 Sorbitan Tetrastearate; PEG-20 and -160 Sorbitan Triisostearate; PEG-18 Sorbitan Trioleate; PEG-40 and -50 Sorbitol Hexaoleate; PEG-30 Sorbitol Tetraoleate Laurate; and PEG-60 Sorbitol Tetrastearate are safe for use as cosmetic ingredients under the present practices of use.

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.

Alade, S. L., R. E. Brown, and A. Paquet, Jr. 1986. Polysorbate 80 and E-Ferol toxicity. *Pediatrics* 77:593–597.

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.

Arechabala, B., P. Rivalland, L. Coiffard, and Y. De Roeck-Holtzhauer. 1995. Compared cytotoxicity of various surfactants tested on human fibroblasts cultures by determining the cellular viability with neutral red. *Cell Biol. Toxicol.* 11:205.

Arni, P. 1985. Studies of the influence of chemicals on mutagen-induced recombination in yeast. *Mutat. Res.* 147:284–285.

Bagley, D. M., P. A. Botham, J. R. Gardner, et al. 1992. Eye irritation: Reference chemicals data bank. *Toxicol. In Vitro* 6:487–491.

Balisteri, W. F., K. E. Bove, N. Kosmetatos, et al. 1985. Distinctive hepatopathy in low birthweight infants in association with E-Ferol infusion. *Pediatr. Res.* 19:212A.

Barnett, J. B. 1981. Immunosuppressive effects of Tween 80 on mice. *Int. Arch. Allergy Appl. Immunol.* 66:229–232.

Barnett, J. B., and R. L. Bryant. 1980. Adjuvant and immunosuppressive effects of retinol and Tween 80 on IgG production in mice. *Int. Arch. Allergy Appl. Immunol.* 63:145–152.

Berry, M., and D. L. Easty. 1993. Isolated human and rabbit eye: models of corneal toxicity. *Toxicol. In Vitro* 7:461–464.

Bonhomme, L., W. Bizi, S. Orbach-Arbouys, J. F. Benoist, and G. Fredj. 1993. Macrophage activity may be modified *in vivo* by galenical excipients. *Int. J. Pharm.* 96:R7–R10.

Borenfreund, E., and O. Borrero. 1984. *In vitro* cytotoxicity assays. Potential alternatives to the Draize ocular allergy test. *Cell Biol. Toxicol.* 1:55–65.

Borenfreund, E., and C. Shopsis. 1985. Toxicity monitored with a correlated set of cell-culture assays. *Xenobiotica* 15:705–712.

Bove, K. E., N. Kosmetatos, K. E. Wedig, et al. 1985. Vasculopathic hepatotoxicity associated with E-Ferol syndrome in low-birth-weight infants. *J. A. M. A.* 254:2422–2430.

Bracher, M., C. Faller, J. Spengler, and C. A. Reinhardt. 1988. Comparison of *in vitro* cell toxicity with *in vivo* eye irritation. *Mol. Toxicol.* 1:561–570.

Bresch, H., and H. Ockenfels. 1977. The influence of Tween surfactants on the development of the sea urchin embryo. *Naturwissenschaften* 64:593–594.

Brown, M. R. W., and B. E. Winsley. 1969. Effect of Polysorbate 80 on cell leakage and viability of *Pseudomonas aeroginosa* exposed to rapid changes of pH, temperature, and tonicity. *J. Gen. Microbiol.* 56:99–107.

Brubaker, C. M., D. H. Taylor, and R. J. Bull. 1982. Effect of Tween 80 on exploratory behavior and locomotor activity in rats. *Life Sci.* 30:1965–1971.

Canterbery, R. C. 1997. Chemical structures of Sorbitan Esters. Correspondence dated 11-17-97. (2 pages.)[2]

Casazza, A. M., G. Pratesi, F. Giuliani, et al. 1978. Enhancement of the antitumor activity of adriamycin by Tween 80. *Tumori.* 64:115–129.

Castro, C. A., J. B. Hogan, K. A. Benson, C. W. Shehata, and M. R. Landauer. 1995. Behavioral effects of vehicles: DMSO, ethanol, Tween-20, Tween-80, and Emulphor-620. *Pharmacol. Biochem. Behav.* 50:521–526.

Chemline. 1996. Entry on PEG Sorbitan Laurate. *Chemline database.* Bethesda, MD: National Library of Medicine (NLM).

Chi, R. K., J. J. Scocca, and P. C. Huang. 1978. An evaluation of Tween 80 effects on the survival and DNA repair in *Escherichia coli* following UV or gamma irradiation. *Mutat. Res.* 49:1–8.

Chitnis, M. P., R. S. Menon, and R. P. Gude. 1984. Effect of Tween 80 on adriamycin cytotoxicity in murine P388 leukemia. *Tumori.* 70:313–315.

Coppinger, W. J., S. A. Brennan, and E. D. Thompson. 1981. Testing of surfactants for mutagenic potential in the L5178Y $TK^{+/-}$ mouse lymphoma assay. *Environ. Mutagen.* 3:320.

Cosmetic Ingredient Review (CIR). 1996. *Special report on the reproductive and developmental toxicity of Ethylene Glycol and its ethers.* Washington, DC: CIR.[2]

Cosmetic Science and Technology On-line. 1997. HLB numbers for surfactants. Oxon, UK: Cotswold Publishing Co. (http://www.cotpubco.demon.co.uk/surfactant.html)

Cosmetic, Toiletry, and Fragrance Association (CTFA). 1998a. Concentration of use information and summary safety data. Unpublished data submitted by CTFA, 4-27-98. (2 pages.)[2]

CTFA. 1998b. Data on PEG Sorbitans/Sorbitols. Unpublished data submitted by CTFA, 1-21-98. (13 pages.)[2]

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

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

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

Crispens, C. G., P. S. Porter, and J. R. J. Sorenson. 1986. Effects of CuDIPS and Tween 80 on SJL/J tumorigenesis. *Biol. Trace Elem. Res.* 10:19–24.

Crispens, C. G., and J. R. J. Sorenson. 1988. Treatment of reticulum cell sarcoma in SJL/J mice with Tween 80. *Anticancer Res.* 8:1341–1344.

Crispens, C. G., and J. R. J. Sorenson. 1990. Combination therapy with CuDIPS, Tween 80, and cyclophosphamide: Effects on survival and tumor incidence in SJL/J mice. *In vivo* 4:321–324.

Crispens, C. G., and J. R. J. Sorenson. 1991. Evaluation of the anticancer activities of Tweens 20, 40, and 60 in SJL/J mice. *Anticancer Res.* 11:407–408.

DeLeo, V. A., D. Hanson, S. Scheide, B. J. Kong, and S. DeSalva. 1989. The effect of surfactants on metabolism of choline phospholipids in human epidermal keratinocytes in culture. *J. Toxicol. Cutan. Ocul. Toxicol.* 8:227–240.

Descotes, J. 1988. Identification of contact allergens: The mouse ear sensitization assay. *J. Toxicol. Cutan. Ocul. Toxicol.* 7:263–273.

Dib, B., and M. Falchi. 1996. Convulsions and death induced in rats by Tween 80 are prevented by capsaicin. *Int. J. Tiss. Reac.* 18:27–31.

Donbrow, M., E. Azaz, and A. Pillersdorf. 1978. Autoxidation of polysorbates. *J. Pharm. Sci.* 67:1676–1678.

Elder, R. L., ed. 1980. Final report of the safety assessment for Acetylated Lanolin and related compounds. *J. Environ. Pathol. Toxicol.* 4:63–92.

Elder, R. L., ed. 1983. Final report on the safety assessment of Isostearic Acid. *J. Am. Coll. Toxicol.* 2:61–74.

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

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.

Elder, R. L., ed. 1986. Final report on the safety assessment of Coconut Oil, Coconut Acid, Hydrogenated Coconut Acid, and Hydrogenated Coconut Oil. *J. Am. Coll. Toxicol.* 5:103–121.

Elder, R. L., ed. 1987. Final report on the safety assessment of Oleic Acid, Lauric Acid, Palmitic Acid, Myristic Acid, and Stearic Acid. *J. Am. Coll. Toxicol.* 6:321–401.

Ema, M., T. Itami, H. Kawasaki, and S. Kanoh. 1988. Teratology study of Tween 60 in rats. *Drug Chem. Toxicol.* 11:249–260.

Epstein, S. S., K. Fujii, J. Andrea, and N. Mantel. 1970. Carcinogenicity testing of selected food additives by parenteral administration to infant Swiss mice. *Toxicol. Appl. Pharmacol.* 16:321–334.

Eschalier, A., J. Lavarenne, C. Burtin, et al. 1988. Study of histamine release induced by acute administration of antitumor agents in dogs. *Cancer Chemother. Pharmacol.* 21:246–250.

Etter, J.-C., and A. Wildhaber. 1985. Biopharmaceutical test of ocular irritation in the mouse. *Fd. Chem. Toxicol.* 23:321–323.

Farkas, W. R., V. Lorch, W. R. Conover, et al. 1991. Polysorbate toxicity in neonatal rats and mice. *Pharmacol. Toxicol.* 68:154–156.

Farrell, R. L. 1974. The effects of Tween 80 on respiratory oncogenesis by benzo(a)pyrene instilled intratracheally in Syrian hamsters. *Vet. Pathol.* 11:449.

Fickentscher, K., A. Kirfel, G. Will, and F. Köhler. 1977. Stereochemical properties and teratogenic activity of some tetrahydropthalimides. *Mol. Pharmacol.* 13:133–141.

Flower, C., B. J. Phillips, and D. Anderson. 1988. Absence of chromosome damage in chinese hamster ovary cells exposed to detergents. *Toxicol. In Vitro* 2:65–66.

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

FDA. 1996. *Food additive safety profile.* Washington, DC: FDA.

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

Fujii, K. 1991. Evaluation of the newborn mouse model for chemical tumorigenesis. *Carcinogens* 12:1409–1505.

Gad, S. C., B. J. Dunn, D. W. Dobbs, O. Reilly, and R. D. Walsh. 1986. Development and validation of an alternative dermal sensitization test: the mouse ear swelling test (MEST). *Toxicol. Appl. Pharmacol.* 84:93–114.

Gadjova, M., J. Jakubovshy, and J. Války. 1993. Delayed effects of neonatal exposure to Tween 80 on female reproductive organs in rats. *Fd. Chem. Toxicol.* 31:183–190.

Gajjar, L., and D. J. Benford. 1987. Irritancy testing in cultured keratinocytes. *Mol. Toxicol.* 1:513–523.

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.

Gaudin, G., S. Robert, G. Yielding, and K. L. Yielding. 1971. DNA repair inhibition: A possible mechanism of action of co-carcinogenesis. *Biochem. Biophys. Res. Commun.* 45:630–636.

Gay, R., M. Swiderek, D. Nelson, and A. Ernesti. 1992. The living skin equivalent as a model *in vitro* for ranking the toxic potential of dermal irritants. *Toxicol. In Vitro* 6:303–315.

Gennaro, A. R., ed. 1990. *Remington's pharmaceutical sciences*, 18th ed., 1314. Easton, PA: Mack Publishing Co.

Gough, W. B., R. H. Zeiler, P. Barreca, and N. El-Sherif. 1982. Hypotensive action of commercial intravenous amiodarone and polysorbate 80 in dogs. *J. Cardiovasc. Pharmacol.* 4:375–380.

Grant, R. L., O. Yao, D. Gabaldon, and D. Acosta. 1992. Evaluation of surfactant cytotoxicity potential by primary cultures of ocular tissues: I. Characterization of rabbit corneal epithelial cells and initial injury and delayed cytotoxicity studies. *Toxicology* 76:153–176.

Grund, F., H. T. Sommerschild, K. A. Kirkebøen, and A. Ilebekk. 1995. Cardiovascular effects of the microsphere suspending agent, Tween 80, in pigs. *Acta Physiol. Scand.* 155:331–332.

Guido, J. A. 1987. Eye irritation tests on two detergents (10% wt/wt in water). *J. Am. Coll. Toxicol.* 11:737.

Hannuksela, M., M. Kousa, and V. Pirila. 1976. Contact sensitivity to emulsifiers. *Contact Dermatitis* 2:201–204.

Hardin, B. D., R. L. Schuler, J. R. Burg, et al. 1987. Evaluation of 60 chemicals in a preliminary developmental toxicity test. *Teratog. Carcinog. Mutag.* 7:29–48.

Harrison, S. D., Jr., A. M. Cusic, and S. M. McAfee. 1981. Tween 80 increases plasma adriamycin concentrations in mice by an apparent reduction of plasma volume. *Eur. J. Cancer* 17:387–389.

Hazardous Substances Database (HSDB). 1996. Entries on PEG-20 Sorbitan Palmitate and PEG-20 Sorbitan Oleate. *HSDB database.* Bethesda, MD: NLM.

Hem, S. L., D. R. Bright, G. S. Banker, and J. P. Pogue. 1974–1975. Tissue irritation evaluation of potential parenteral vehicles. *Drug Dev. Commun.* 1:471–477.

Hendy, R. J., K. R. Butterworth, I. F. Gaunt, and I. S. Kiss. 1978. Long-term toxicity study of sorbitan monostearate (Span 60) in mice. *Fd. Cosmet. Toxicol.* 16:527–534.

Igarashi, H., and A. M. Northover. 1987. Increases in opacity and thickness induced by surfactants and other chemicals in the bovine isolated cornea. *Toxicol. Lett.* 39:249–254.

Inoue, K., T. Sunakawa, and T. Takayama. 1980. Studies of *in vitro* cell transformation and mutagenicity by surfactants and other compounds. *Fd. Cosmet. Toxicol.* 18:289–296.

Ishidate, M., Jr., and S. Odashima. 1977. Chromosome tests with 134 compounds on Chinese hamster cells *in vitro*—a screening for chemical carcinogens. *Mutat. Res.* 48:337–354.

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

Jacobs, G. A., and M. A. Martens. 1989. An objective method for the evaluation of eye Irritation *in vivo*. *Fd. Chem. Toxicol.* 27:255–258.

Kada, T., K. Hirano, and Y. Shirasu. 1980. Screening of environmental chemical mutagens by the rec-assay system with *Bacillus Subtilis*. *Chem. Mutag.* 6:149–173.

Kavlock, R. J., R. D Short, Jr., and N. Chernoff. 1987. Further evaluation of an *in vivo* teratology screen. *Teratog. Carcinog. Mutag.* 7:7–16.

Kawachi, T., T. Komatsu, T. Kada, et al. 1980a. Results of recent studies on the relevance of various short-term screening tests in Japan. In *Appl. Methods Oncol.* 3:253–267.

Kawachi, T., T. Yahagi, T. Kada, et al. 1980b. Cooperative programme on short-term assays for carcinogenicity in Japan. *I.A.R.C. Sci. Publ.* 27:323–330.

Kocher-Becker, U., and W. Kocher. 1981. Thalidomide-like malformations caused by a Tween surfactant in mice. *Z. Naturforsch.* 36c:904–906.

Köhler, V. F., and H. Koch. 1974. Teratologische untersuchung der thalidomid-ähnlichen verbindungen K-2004 und K-2604 an maus und ratte. *Arzneim. Forsch.* 24:1616–1619.

Kubis, A., R. Witek, Z. Olszewski, and S. Krupa. 1979. The cytotoxic effect of Tween 80 on Ehrlich ascites cancer cells in mice. *Pharmazie* 34:745–746.

Levinson, R. S., L. V. Allen, Jr., and B. H. Sung. 1978. Effect of polysorbate 80 on *Escherichia coli* cultures. *Can. J. Pharm. Sci.* 13:48–50.

Lewis, R. J., Sr. 1993a. *Hawley's condensed chemical dictionary*, 12th ed., 941, 1077. New York: Van Nostrand Reinhold.

Lewis, R. J., Sr. 1993b. *Hazardous chemicals desk reference*, 3rd ed., 1057. New York: Van Nostrand Reinhold.

Lorch, V., D. Murphy, L. R. Hoersten, et al. 1985. Unusual syndrome among premature infants: Association with a new intravenous vitamin E product. *Pediatrics* 75:598–602.

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. *Fd. Chem. Toxicol.* 24:191–200.

Marsh, R. J., and D. M. Maurice. 1971. The influence of non-ionic detergents and other surfactants on human corneal permeability. *Exp. Eye Res.* 11:43–48.

Martinez, T. T., and K. Brown. 1991. Oral and pulmonary toxicology of the surfactant used in Roundup herbicide. *Proc. West Pharmacol. Soc.* 34:43–46.

Maurice, D. M., and D. Brooks. 1995. The permeability of the mouse cornea as a test for acute ocular toxicity. *In Vitro Toxicol.* 8:113–120.

McKean, D. L. 1992. The neonatal toxicity of polysorbate. *Diss. Abstr. Int. B* 53:1320.

McKean, D. L., and A. J. Pesce. 1985. Determination of polysorbate in ascites fluid from a premature infant. *J. Anal. Toxicol.* 9:174–176.

McKean, D. L., A. J. Pesce, and W. Koo. 1987. Analysis of polysorbate and its polyoxyethylated metabolite. *Anal. Biochem.* 161:348–351.

Meise, W., H. Ockenfels, and F. Köhler. 1973. Teratogische prüfung der hydrolysenprodukte des thalldomids. Experientia 29:423–424.

Menon, R., M. Chitnis, M. Adwankar, V. Basrur, and A. Amonkar. 1984. Tween-80 alters natural resistance of sarcoma-180 to bouvardin (NSC 259968). *Tumori.* 70:23–27.

Mezei, M., R. W. Sager, W. D. Stewart, and A. L. Deruyter. 1966. Dermatitic effect of nonionic surfactants. I. Gross, microscopic, and metabolic changes in rabbit skin treated with nonionic surface-active agents. *J. Pharm. Sci.* 55:584–590.

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

Morris, W. E., and S. C. Kwan. 1983. Use of the rabbit ear model in evaluating the comedogenic potential of cosmetic ingredients. *J. Soc. Cosmet. Chem.* 34:215–225.

Nagami, K., and E. Maki. 1993. *In vitro* cytotoxicity test for estimating non-ocular irritation dose of ophthalmic solutions. *Cell Biol. Toxicol.* 9:107–118.

National Toxicology Program (NTP). 1992a. *Final report on the Developmental Toxicity of Polyoxyethylene Sorbitan Monolaurate (CAS No. 9005-64-5) in Sprague-Dawley (CD (Trade Name)) rats on gestational days 6 through 15.* Laboratory Supplement. Sections 1-7A. NTIS No. PB93-123818.

NTP. 1992b. *Toxicology and carcinogenesis studies of Polysorbate 80 (CAS No. 9005-65-6) in F344/N Rats and B6C3F, mice (feed studies).* NTP Technical Report No. 415. Research Triangle Park, NC: NTP.

NTP. 1992c. *Final report on the developmental toxicity of Polyoxyethylene Sorbitan Monolaurate (CAS No. 9005-64-5) in Sprague-Dawley (CD (Trade Name)) rats on gestational days 6 through 15* (includes Appendix). NTIS No. PB93-123800.

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

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

North-Root, H., F. Yackovich, J. Demetrulias, et al. 1992. Evaluation of an in vitro cell toxicity test using rabbit corneal cells to predict the eye irritation potential of surfactants. *Toxicol. Lett.* 14:207–212.

Oberie, R. L., T. J. Moore, and D. A. P. Krummel. 1995. Evaluation of mucosal damage of surfactants in rat jejunum and colon. *J. Pharmacol. Toxicol. Methods* 33:75–81.

Odashima, S. 1976. The cooperative development in Japan of methods for screening chemicals for carcinogenicity. *I.A.R.C. Sci. Publ.* 12:61–75.

Ohsumi, T., M. Yonezawa, T. Aizawa, et al. 1979. Basic studies on digestion and absorption in the small intestine: Long-term effects of the detergents on rat intestinal mucosa. *J. Clin. Electron Microsc.* 12:5–6.

Pape, W. J. W., U. Pfannenbecker, and U. Hoppe. 1987. Validation of the red blood cell test system as in vitro assay for the rapid screening of irritation potential of surfactants. *Mol. Toxicol.* 1:525–536.

Parfett, C. L. J. 1992. Induction of proliferin gene expression by diverse chemical agents that promote morphological transformation in C3H/10T1/2 cultures. *Cancer Lett.* 64:1–9.

Paschall, H. D. 1964. Effects of feeding emulsifiers on reproduction of C57BL/6 mice. *Diss. Abstr.* 24:1764–1765.

Pasche-Koo, F., P.-A. Piletta, N. Hunziker, and C. Hauser. 1994. High sensitization rate to emulsifiers in patients with chronic leg ulcers. *Contact. Dermatitis* 31:226–228.

Pazzaglia, M., C. Vincenzl, M. Spiller, and A. Tosti. 1995. Contact sensitivity to Atlas G-1441. *Contact Dermatitis* 33:208.

Perkins, M. A., R. Osborne, and G. R. Johnson. 1996. Development of an in vitro method for skin corrosion testing. *Fundam. Appl. Toxicol.* 31:9–18.

Pesce, A. J., and D. L. McKean. 1989. Toxic susceptibilities in the newborn with special consideration of polysorbate toxicity. *Ann. Clin. Lab. Sci.* 19:70–73.

Rachui, S. R., W. D. Robertson, M. A. Duke, and J. Heinze. 1994. Predicting the ocular irritation potential of surfactants using the in vitro Skin^2 model ZK1200. *J. Toxicol. Cutan. Ocul. Toxicol.* 13:215–220.

Radian Corporation. 1991. Entry on Polysorbate 80. *NTP chemical repository.* Washington, DC: National Institutes of Health. http://www.ntp-db.niehs.nih.gov/NTP_Reports/NTP_Chem_H&S/NTP_Chem9/Radian 9005-65-6.txt

Rivera, A., K. M. Abdo, J. R. Bucher, et al. 1990. Toxicity studies of intravenous vitamin E in newborn rabbits. *Dev. Pharmacol. Ther.* 14:231–237.

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

Roguet, R., C. Cohen, K. G. Dossou, and A. Rougier. 1994. Episkin, a reconstituted human epidermis for assessing in vitro the irritancy of topically applied compounds. *Toxicol. In Vitro* 8:283–291.

Sabir, M., M. Singh, and N. K. Bhide. 1972. Spasmolytic effect of polysorbates (Tweens) 80 and 20 isolated tissues. *Ind. J. Physiol. Pharmacol.* 16:193–199.

Saffioti, U., and P. Shubik. 1963. Studies on promoting action in skin carcinogenesis. *Natl. Cancer Inst. Monogr.* 10:489–507.

Sakane, T., C. Tanaka, A. Yamamoto, et al. 1989. The effect of polysorbate 80 on brain uptake and analgesic effect of D-kyotorphin. *Int. J. Pharm.* 57:77–83.

Sarpotdar, P. P., and J. L. Zatz. 1986. Evaluation of penetration enhancement of lidocaine by nonionic surfactants through hairless mouse skin *in vitro*. *J. Pharm. Sci.* 75:176–181.

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.

Schmid, B. P., A. Trippmacher, and A. Bianchi. 1983. Validation of the whole-embryo culture method for *in vitro* teratogenicity testing. *Dev. Toxicol. Environ. Sci.* 11:563–566.

Schulz, C. O. 1974. Acute lung injury from the interaction of a plasticizer di-2-ethylhexyl phthalate and a polysorbate surfactant Tween 80. *Fed. Proc.* 33:234.

Scott, B. R., and T. Alderson. 1973. Does "Tween 80" interfere with induction of mutagenic and lethal damage in *Aspergillus? Microb. Genet. Bull* 35:7.

Scott, W. J., R. Fradkin, and J. G. Wilson. 1977. Non-confirmation of thalidomide induced teratogenesis in rats and mice. *Teratology* 16:333–336.

Setälä, H. 1956. Tumor promoting and co-carcinogenic effects of some non-ionic lipophillic-hydrophillic (surface active) agents: An experimental study on skin tumors in mice. *Acta Pathol. Microbiol. Scand.* (supp. 115):1–93.

Shirai, T., K. Imaida, S. Fukushima, R. Hasegawa, M. Tatematsu, and N. Ito. 1982. Effects of NaCl, Tween 60, and a low dose of N-ethyl-N′-nitro-N-nitrosoguanidine on gastric carcinogenesis of rat given a single dose of N-methyl-N′-nitro-N-nitrosoguanidine. *Carcinogenesis* 3:1419–1422.

Shivji, G. M., L. Segal, R. C. McKenzie, and D. N. Sauder. 1994. Cutaneous toxicity of surfactants in normal human keratinocytes assessed by cytotoxicity, arachidonic acid release, and regulation of interleukin-1 α mRNA. *Toxicol Methods* 4:193–203.

Siegel, I. A., and H. P. Gordon. 1985. Effects of surfactants on the permeability of canine oral mucosa in vitro. *Toxicol. Lett.* 26:153–157.

Siegel, I. A., and H. P. Gordon. 1986. Surfactant-induced alterations of permeability of rabbit oral mucosa *in vitro*. *Exp. Mol. Pathol.* 44:132–137.

Smolinske, S. C. 1992. *CRC handbook of food, drug, and cosmetic excipients*, 295–301. Boca Raton, FL: CRC Press.

Stavrovskaya, A. A., T. V. Potapova, V. A. Rosenblat, and A. S. Serpinskaya. 1975. The effect of non-ionic detergent Tween 80 on colcemid-resistant transformed mouse cells. *Int. J. Cancer* 15:665–672.

Tachon, P., K. Cotovio, K. G. Dossou, and M. Prunieras. 1989. Assessment of surfactant cytotoxicity: Comparison with the Draize eye test. *Int. J. Cosmet. Sci.* 11:233–243.

Tagesson, C., and C. Edling. 1984. Influence of surface-active food additives on the integrity and permeability of rat intestinal mucosa. *Fd. Chem. Toxicol.* 22:861–864.

Tosti, A., L. Guerra, R. Morelli, and F. Bardazzi. 1990. Prevalence and sources of sensitization to emulsifiers: A clinical study. *Contact Dermatitis* 23:68–72.

Trosko, J. E., L. P. Yotti, B. Dawson, and C.-C. Chang. 1981. In vitro assay for tumor promoters. *Short-Term Tests for Chemical Carcinogens* 420–427.

Trosko, J. E., L. P. Yotti, S. T. Warren, G. Tsushimoto, and C.-C. Chang. 1982. Inhibition of cell-cell communication by tumor promoters. *Carcinogens* 7:565–585.

Uluoglu, C., P. Korkusuz, O. Uluoglu, and H. Zengil. 1996. Tween 80 and endothelium: Functional reduction due to tissue damage. *Res. Commun. Pathol. Pharmacol.* 91:173–183.

Unichema International. 1996. Polyalcohol Isostearate Esters: Safety evaluation. Unpublished data submitted by CTFA, 5-98. (9 pages.)^2

van de Sandt J. J. M., A. A. J. J. L. Rutten, and H. B. W. M. Koëter. 1993. Cutaneous toxicity testing in organ culture: Neutral red uptake and reductio of tetrazolium salt (MMT). *Toxicol. In Vitro* 7:81–86.

Vanparys, P., D. Deknudt, M. Sysmans, et al. 1993. Evaluation of the bovine corneal opacity-permeability assay as an *in vitro* alternative to the Draize eye irritation test. Toxicol. *In Vitro* 7:471–476.

Wenninger, J. A., R. C. Canterbery, and G. N. McEwen, Jr., eds. 2000. *International cosmetic ingredient dictionary and handbook*, 8th ed. Vol. 1–3. Washington, DC: CTFA.

Wickramaratne, G. A. de S. 1987. The Chernoff-Kavlock assay: Its validation and application in rats. *Teratog. Carcinog. Mutag.* 7:73–83.

Yang, W., and D. Acosta. 1994. Cytotoxicity potential of surfactant mixtures evaluated by primary cultures of rabbit corneal epithelial cells. *Toxicol. Lett.* 70:309–318.

Yao, W., and D. Acosta. 1992. Surfactant cytotoxicity potential evaluated with primary cultures of ocular tissues: A method for the culture of rabbit conjunctival epithelial cells and initial cytotoxicity studies. *Toxicol. Methods* 2:199–218.

York, M., H. A. Griffiths, E. Whittle, and D. A. Basketter. 1996. Evaluation of a human patch test for the identification and classification of skin irritation potential. *Contact Dermatitis* 34:204–212.