Final Report on the Safety Assessment of PEG-5, -10, -16, -25, -30, and -40 Soy Sterol[1]

PEGs Soy Sterol are PEG (polyethylene glycol) derivatives of soybean oil sterols used in a variety of cosmetic formulations as nonionic surfactants; emulsifying, skin conditioning, cleansing and solubilizing agents; appearance and consistency modifiers; emollients; viscosity control agents; and pigment dispersion agents. The concentrations at which these ingredients are used as a function of the particular chain length polymer, but the maximum concentration recently reported was 2% in mascara and eyeliner. PEGs Soy Sterol are produced by the reaction of the soy sterol hydroxyl with ethylene oxide. In general, ethoxylated fatty acids can contain 1,4-dioxane as a byproduct of ethoxylation. The soy sterols include γ-sitosterol, campesterol, stigmasterol, and possibly β-sitosterol. The amount of β-sitosterol, if any, is not known. Impurities include sterol hydrocarbons and cholesterol (4–6%) and triterpine alcohols, keto-steroids, and other steroid-like substances (4–6%). Because PEGs are an underlying structure in PEGs Soy Sterols, a previous assessment of PEGs was considered. The acute oral LD_{50} in rats of PEGs 5–25 Soy Sterol was >10 g/kg. The acute dermal LD_{50} of a liquid eyeliner containing 2% PEG-5 Soy Sterol was >2 g/kg. PEGs 5–25 Soy Sterol were not primary irritants in rabbits when applied undiluted. Undiluted PEG-5 Soy Sterol did not cause sensitization in guinea pigs. PEGs Soy Sterol did not produce ocular toxicity in rabbits. PEG-5 Soy Sterol was negative in the Ames mutagenicity test, with or without metabolic activation. PEG-5 Soy Sterol, at concentrations up to 2% in formulation, did not cause dermal or ocular irritation, dermal sensitization, or photosensitization in clinical studies. Subcutaneous injections of β-sitosterol reduced sperm concentrations and fertility. Sulfates of β-sitosterol acted as abortifacients in female rats and rabbits. 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 PEGs Soy Sterol, there is no likelihood of ethylene glycol or its alkyl ethers being present, and the soybean oil sterol ethers in this ingredient are chemically different from the ethylene glycol alkyl ethers of concern. PEGs are not carcinogenic, although sensitization and nephrotoxicity were observed in burn patients treated with a PEG-based cream. No evidence of systemic toxicity or sensitization was found in studies with intact skin. Because of the possible presence of 1,4-dioxane reaction product and unreacted ethylene oxide residues, it was considered necessary to use appropriate procedures to remove these from PEGs Soy Sterol before blending them into cosmetic formulations. Based on the systemic toxicity and sensitization seen with PEGs applied to damaged skin, PEGs Soy Sterol should not be used in cosmetic products applied to damaged skin. Overall, the available data on PEGs Soy Sterols and related compounds were not adequate to complete a safety assessment. Of particular concern is the reproductive toxicity of free phytosterols. Additional data needs include: (1) impurities data; (2) current concentration of use; (3) genotoxicity in a mammalian system of PEG-5 Soy Sterol; if positive, then a 2-year dermal carcinogenicity study using National Toxicology Program (NTP) methods is needed; (4) skin sensitization and irritation in humans at concentration of use; and (5) dermal absorption of PEG-5 Soy Sterol; if significantly absorbed, then both a 28-day dermal toxicity study and reproductive and developmental toxicity data may be needed (alternatively, data showing that dietary intake results in the release/availability of phytosterols would suffice). Until these data are provided, the available data are insufficient to support the safety of PEG-5, -10, -16, -25, -30, and -40 Soy Sterol in cosmetic products.

INTRODUCTION

PEG-5, -10, -16, -25, -30, and -40 Soy Sterol are polyethylene glycol (PEG) derivatives of soybean oil sterols that function as nonionic surfactants and emulsifying agents in cosmetic formulations. Polyethylene glycol has been reviewed previously by the Cosmetic Ingredient Review (CIR) Expert Panel and the Final Report has been published. The following conclusion was made:

> 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).

Because there is limited data specifically on the PEGs Soy Sterol ingredients, safety test and other (e.g., impurities) data on Polyethylene Glycol have been included along with similar data on phytosterols that make up the soy sterol component of PEGs Soy Sterol ingredients. In each section, information on PEGs Soy Sterol is provided first, followed by information on Polyethylene Glycol and phytosterols. These data are considered relevant to assessing the safety of PEGs Soy Sterol ingredients.

CHEMISTRY

Definition and Structure

PEG-*n* Soy Sterol is a PEG derivative of sterols found in soybean oil where *n* = the average number of moles of ethylene

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oxide (Wenninger, Canterbery, and McEwen 2000; Nikitakis and McEwen 1990). These phytosterols (generic term) are structurally similar to cholesterol and mainly consist of γ-sitosterol ($C_{29}H_{50}O$: MW 414.69 Da), campesterol ($C_{28}H_{48}O$: MW 400.66 Da), and stigmasterol ($C_{29}H_{48}O$: MW 412.67 Da) (Applewhite 1985; Budavari 1989; Tyle and Frank 1991). β-Sitosterol has also been detected (General Mills, Inc. 1979). Campesterol and sitosterol are structurally identical to cholesterol except for side chain substitution of a methyl or ethyl group at the C24 position, respectively. Stigmasterol has an additional double bond at C22 (Heinemann, Axtmann, and von Bergmann 1993). Their general structures are depicted in Figure 1.

PEG-*n* Soy Sterol is also known as PEG-*n* Soya Sterol (Wenninger, Canterbery, and McEwen 2000) or Polyoxyethylene (*n*) Soya Sterol. A synonym for PEG-16 Soy Sterol is Soyasterole-PEG-16-Ether (Baade and Mueller-Goymann 1994). Additionally, PEG-10 and -40 Soy Sterol go by the names Polyethylene Glycol 500 Soy Sterol. (Wenninger, Canterbery, and McEwen 2000).

Chemical and Physical Properties

PEG-5 and -10 Soy Sterols are soft, amber-colored, waxy solids with little or no odor (Lundmark, Chun, and Melby 1976; Nikitakis and McEwen 1990). PEG-16 and -25 Soy Sterols are ivory-colored, hard waxes (Lundmark, Chun, and Melby 1976). The compounds are more hydrophilic as the degree of ethoxylation increases, although the melting points (Tyle and Frank 1991) and interfacial tensions decrease linearly. The polyethylene chains in the PEG Soy Sterols form conical micelles with the base of the cone at the exterior of the micelle (Lundmark, Chun, and Melby 1976). Physical and chemical properties of PEG Soy Sterols are summarized in Table 1.

PEG-5 Soy Sterol is soluble in ethyl alcohol and hot isopropyl myristate. It is also dispersible in water. PEG-5 Soy Sterol melts in the range of 74 to 88°C, and has a pH of 5.0 to 7.0 in a 1% aqueous dispersion at 25°C. The compound has an 80 to 110 hydroxyl value (Nikitakis and McEwen 1990). The apparent hydrophile-lipophile balance (HLB) is 5 (Lundmark, Chun, and Melby 1976). The HLB illustrates the simultaneous relative attraction of the compound for both water and oil, and identifies PEG-5 Soy Sterol as being oil-dispersible (Balsam and Sagarin 1974).

The HLB for PEG-10 Soy Sterol is 12 (Lundmark, Chun, and Melby 1976); PEG-10 Soy Sterol is dispersible in mineral and vegetable oils at high temperatures, soluble in isopropyl myristate (Lundmark, Chun, and Melby 1976), and, in water, forms a translucent dispersion (Balsam and Sagarin 1974; Lundmark, Chun, and Melby 1976). PEG-10 Soy Sterol has a melting range of 73 to 80°C, a pH of 4.5 to 7.0 (in 1% aqueous dispersion at standard temperature), and a hydroxyl value of 60 to 90 (Nikitakis and McEwen 1990), although Lundmark, Chun, and Melby (1976) give a melting point range of 55 to 58°C.

PEG-16 Soy Sterol melts at 46 to 50°C and has an HLB of 15 (Lundmark, Chun, and Melby 1976). Its hydroxyl value is 70. The critical micelle concentration (CMC) for this compound is 0.22%. PEG-16 Soy Sterol contains enough ethylene oxide adducts to be soluble in water (Lundmark, Chun, and Melby 1976).

PEG-25 Soy Sterol melts at 44 to 48°C (Lundmark, Chun, and Melby 1976; Tyle and Frank 1991) and has an HLB of 17 (Lundmark, Chun, and Melby 1976). The hydroxyl value of the compound is 55. PEG-25 Soy Sterol has a CMC of 0.46%. Like PEG-16 Soy Sterol, this compound is water soluble (Lundmark, Chun, and Melby 1976).

Method of Manufacture

Soy Sterol is isolated from soybean oil distillates in a saponification process in which the phytosterols are separated from the fatty acids by extraction with a fat solvent. The phytosterols in the resulting extract are separated from the tocopherols in the mother liquor, and then purified and/or separated into the constituent sterols.

PEG-*n* Soy Sterol is formed from the reaction of the soy sterol hydroxyl with *n* moles of ethylene oxide (Lundmark, Chun, and Melby 1976).

Analytical Methods

The PEGs Soy Sterol can be determined by nuclear magnetic resonance and infrared spectroscopy. In the presence of an amphoteric surfactant, stabilized oil-in-water emulsions are observed, with the association complexes appearing to form liquid crystalline phases at the oil-water interfaces when viewed by polarized light and freeze-fracture electron microscopy (Tyle and Frank 1990). The temperature at which the liquid crystals form was inversely proportional to the degree of hydrophilicity of the phytosterol in question (Tyle and Frank 1991).

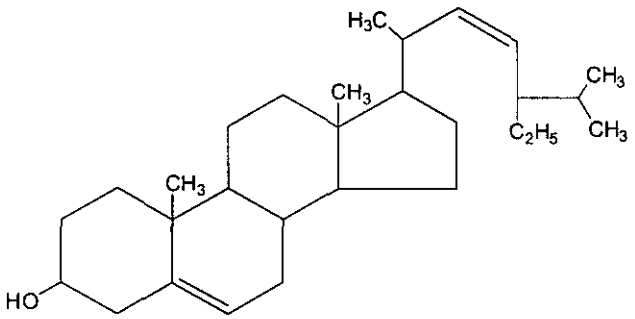
Baade and Mueller-Goymann (1994) separated PEG-16 Soy Sterol from the surface active local drug lidocaine using gel permeation chromatography, ultraviolet (UV) spectroscopy, and nuclear magnetic resonance spectroscopy. A 40% PEG-16 Soy Sterol aqueous solution is a highly viscous liquid that is isotropic in polarized light microscopy. The critical micelle concentration of PEG-16 Soy Sterol is approximately 2 mg/L. When analyzed by UV spectroscopy, the compound absorbs at 270 nm and 293 nm in a ratio of ~1.73.

Impurities

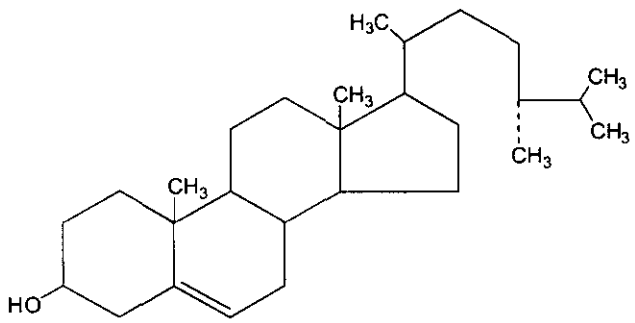
Polyethylene Glycol

Silverstein et al. (1984) reported that PEG-6 may contain small amounts of monomer and dimers. The amounts were not quantified.

Peroxides, formed as a result of autoxidation, are found in PEG-32 and PEG-75 (Hamburger, Azaz, and Donbrow 1975).



Stigmasterol (Budavari, 1989)



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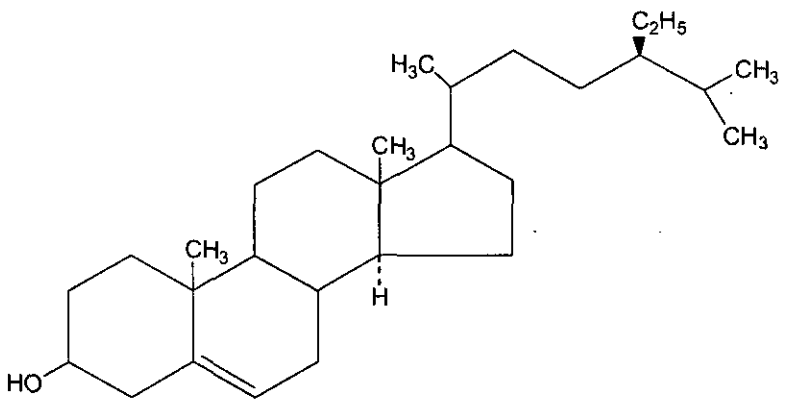
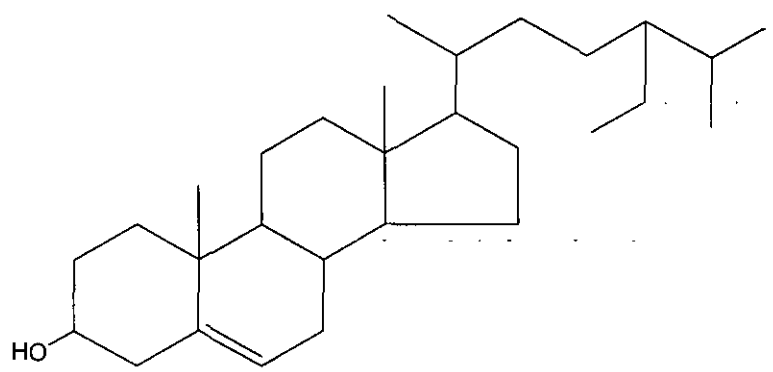


γ-Sitosterol (Budavari, 1989)



β-Sitosterol (Lewis, 1993)

FIGURE 1

Soy Sterols found in PEG Soy Sterols. These mainly consist of γ-Sitosterol, campesterol, and stigmasterol, but β-sitosterol has also been detected.

TABLE 1
Physical properties of PEG-*n* Soy Sterol

Description	PEG -5	PEG -10	PEG -16	PEG -25	Reference
Appearance	Soft, waxy solid; little or no odor; light to medium amber	Soft, waxy solid; little or no odor; light to medium amber	Hard wax; ivory-colored	Hard wax; ivory-colored	Lundmark, Chun, and Melby 1976; Nikitakis and McEwen 1990
Solubility	Soluble in ethyl alcohol and hot isopropyl myristate; dispersible in water	Dispersible in mineral and vegetable oils; soluble in isopropyl myristate; forms translucent dispersion in water	Water soluble	Water soluble	Lundmark, Chun, and Melby 1976; Nikitakis and McEwen 1990
Melting point	74–88°C	73–80°C (55–58°C)	46–50°C	44–48°C	Lundmark, Chun, and Melby 1976; Nikitakis and McEwen 1990; Tyle and Frank 1991
Hydrophile-lipophile balance	5	12	15	17	Lundmark, Chun, and Melby 1976
pH (1% aqueous dispersion at 25°C)	5.0–7.0	4.5–7.0	—	—	Lundmark, Chun, and Melby 1976; Nikitakis and McEwen 1990
Hydroxyl value	80–110	60–90	70	55	Lundmark, Chun, and Melby 1976; Nikitakis and McEwen 1990
Critical micelle concentration	—	—	0.22%	0.46%	Lundmark, Chun, and Melby 1976

The amount of peroxide in PEGs is 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 PEGs, 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 thought to be organic peroxides rather than hydrogen peroxide (McGinity, Hill, and La Via 1975).

Ethoxylated surfactants can contain 1,4-dioxane, a by-product of ethoxylation (Robinson and Ciurczak 1980), if steps are not taken to remove it. 1,4-Dioxane is a known animal carcinogen (Kociba et al. 1974; Hoch-Ligeti, Argus, and Arcos 1970; Argus, Arcos, and Hoch-Ligeti 1965). In the CIR safety assessment of the PEGs Stearate, the cosmetic industry reported that it is aware that 1,4-dioxane may be an impurity in PEGs and, thus, uses additional purification steps to remove it from the ingredient before blending into cosmetic formulations (Elder 1983).

Phytosterols

Refined plant sterols contain approximately 88% total soy sterol content. Of that percentage, 56% is γ-sitosterol, 28% is campesterol, and 4% is stigmasterol. Other compounds isolated with the phytosterols are 4% to 6% sterol hydrocarbons and cholesterol and 4% to 6% triterpene alcohols, keto-steroids, and other steroidlike substances (Lundmark, Chun, and Melby 1976). Soybean oil that had been alkali-refined typically contained (per 100 mg oil) 0.446 mg total sterol and 0.287 mg free sterol. The ratio of esterified to free sterol was 0.55 (Swern 1979).

Analyses of various lots of soy sterols for pesticide residues were negative for a number of pesticides, including polychlorinated biphenyl (PCB), DDE, DDT, Malathion, and β-hexachloride (General Mills, Inc. 1979).

USE

Cosmetic

PEGs 5-40 Soy Sterol serve as surfactants and emulsifying agents in cosmetic formulations. PEG-5 and -10 Soy Sterol function as skin-conditioning agents and PEG-40 Soy Sterol is used as a cleansing and solubilizing agent (Wenninger, Canterbery, and McEwen 2000). These compounds also are used as appearance and consistency modifiers, emollients, viscosity control agents, and pigment dispersion agents (Lundmark, Chun, and Melby 1977).

Table 2 is a summary of the product formulation data submitted to the Food and Drug Administration (FDA) in 1996. PEG-5, -10, -16, -25, and -40 Soya Sterol were used in 41, 35, 15, 5, and 1 cosmetic formulations, respectively. PEG-30 Soya Sterol was not used (FDA 1996). Concentration of use data are no longer required to be submitted to the FDA by the cosmetics industry, but data from 1984 stated that PEG-5 and -10 Soy Sterol were used at concentrations up to 5%, whereas the maximum concentration of use for both PEG-16 and -25 was 1% (FDA 1984).

PEG-5 Soy Sterol was used at concentrations of 2% in mascara and eyeliner. In addition, 0.75% PEG-5 Soy Sterol and 1.5% PEG-25 Soy Sterol were reported used in a liquid makeup foundation (Cosmetic, Toiletry, and Fragrance Association [CTFA] 1997). Toxicology studies of these cosmetic formulations are summarized in this report.

Noncosmetic

In 1982, polyoxyethylene adducts of mixed phytosterols (with 5–26 moles average polyoxyethylene content) were exempted from tolerance requirements by the Environmental Protection Agency (EPA) when used as surfactants or related surfactant adjuvants in pesticide formulations applied to growing crops (EPA 1982; Rothschild 1990).

GENERAL BIOLOGY OF PHYTOSTEROLS

Campesterol, sitosterol, and stigmasterol are the most frequently occurring plant sterols (Kallianos et al. 1963). Approximately 0.25 to 0.5 g of plant sterols are consumed each day in a typical diet (Sabine 1977; Heinemann, Axtmann, and von Bergmann 1993). As a comparison, up to 1.2 g cholesterol, which is derived from animal fat (Tso and Fujimoto 1991), was consumed (Sabine 1977). Plant sterols account for 20% to 25% of total dietary sterols (Heinemann, Axtmann, and von Bergmann 1993). These sterols are also found in cigarette and tobacco smoke as free sterols (Kallianos et al. 1963).

TABLE 2
Product formulation data for PEG-*n* Soy Sterol (FDA 1996)

Product category	Total no. of formulations in category	Total no. of formulations containing ingredient PEG				
		-5	-10	-16	-25	-40
Eyeliner	533	1				
Mascara	218	5				
Other eye makeup preparations	136		1	3		
Hair conditioners (noncoloring)	715	3	1			
Shampoos (noncoloring)	972		1			
Blushers (all types)	277	1	1			
Foundations	355	1	1	1	2	
Rouges	30		1			
Aftershave lotion	268	1	1			
Cleansing	820	1	14	3		1
Face and neck (excluding shaving)	300	5				
Body and hand (excluding shaving)	1012	4	3			
Moisturizing	942	8	5	1		
Night	226		1			
Paste masks (mud packs)	300	1	2		3	
Other skin care preparations	810	8				
Suntan gels, creams, and lotions	196	2	3			
1996 totals		**41**	**35**	**8**	**5**	**1**

Phytosterols affect plant membrane structure and water permeability (Hennessey 1992). Membrane fluidity is inversely related to the amount of sterol found in the membrane (Sabine 1977). Phytosterols are commonly found in animal cell membranes following dietary uptake. In general, sterols intercalate into membrane bilayers and align themselves perpendicularly to the plane of the membrane with the 3′ OH facing the water interface. The aliphatic side chain extends into the hydrophobic core to interact with the fatty acid side chains of phospholipids and integral membrane proteins. The phytosterols are less water soluble than cholesterol. Sitosterol and campesterol order bilayer acyl chains most effectively, followed by cholesterol and stigmasterol (Hennessey 1992).

Absorption, Metabolism, Distribution, and Excretion

Polyethylene Glycol

Gastrointestinal absorption of PEGs 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).

Phytosterols

In general, ingested sterols are emulsified in the stomach, where lipid material from lipoprotein complexes is released. The coarse emulsion enters the duodenum of the small intestine, and the emulsion is solubilized with pancreatic juice and bile. Bile salts become conjugated with fatty acids, monoglycerides, dissolved sterols, and other molecules in the jejunum to form mixed micelles. Sterol ester bonds become hydrolyzed until only free sterol remains. Micellar solutions of lipids are very rapidly absorbed, and represent the major pathway of absorption for sterols and other fats (Sabine 1977). For example, incorporation of sterols into cholic acid micelles was 34%, 30%, 23%, and 15% for campesterol, sitosterol, cholesterol, and stigmasterol, respectively (Hennessey 1992). Micelles release sterols to the cells of the intestinal wall. Absorbed sterols are mixed with cholesterol synthesized within the intestinal cells. Before release from mucosal cells, the sterols are esterified. The resulting esters are transported from the intestine via the lymph (Sabine 1977).

Once consumed, phytosterols only enter the body via intestinal absorption. As the absorption rate for the plant sterols is usually less than 5% of dietary concentrations in humans (Sabine 1977; General Mills, Inc. 1979; Ling and Jones 1995), approximately 95% of dietary phytosterols enters the colon (Ling and Jones, 1995). Saturated sterols are virtually not absorbed (Vanhanen and Miettinen 1992).

When cholesterol and phytosterols were simultaneously administered, only cholesterol could be isolated from the lymph duct, demonstrating that the phytosterols had not been significantly absorbed. Phytosterols experimentally injected subcutaneously into dogs were not esterified or metabolized. Instead, the plant sterols were treated like inert, foreign materials. Cholesterol, when similarly injected, was readily esterified (Lange 1950). In other studies, sitosterols did not accumulate or deposit in tissues (Gould 1955; Gould et al. 1969).

Freshly absorbed sterols are transported into plasma (Sabine 1977). Dietary supplementation with phytosterols can increase their serum concentrations until the sterols represent 10% of total serum sterols (Hennessey 1992; Heinemann, Axtmann, and von Bergmann 1993). Ling and Jones (1995) reported that 0.3 to 1.7 mg/dl of phytosterols were found in human serum under normal conditions after daily phytosterol consumption of 160 to 360 mg/day.

During metabolism and excretion, the sterol rings generally remain intact; double bonds, constituent groups, and side chains are often added, removed, or modified. The largest proportion of sterol in the body is converted to bile acids (Sabine 1977). In feeding studies, approximately 20% of absorbed β-sitosterol was converted to bile acids (cholic and chenodeoxycholic) in humans (General Mills, Inc. 1979). Boberg et al. (1990) reported that C21 bile acids were major metabolites of sitosterol in mammals. Sitosterol was apparently not converted into C24 bile acids in humans (Boberg, Einarsson, and Björkhem 1990). Conversion of campesterol into bile acids was reported in rats (Boberg et al. 1990). Absorbed phytosterols not converted to normal bile acids were excreted as the free sterol (General Mills, Inc. 1979).

5,6-Epoxides were formed in the liver from β-sitosterol. β-sitosterol was metabolized to cortisol by the adrenal glands and to various steroid hormones by the testes. In rats, up to 5% of adrenal gland sterols can be of plant origin, and other tissues can contain large amounts of plant sterols (Sabine 1977).

Phytosterols can act as plant hormone and hormone precursors (Hennessey 1992). Other metabolites include steroid hormones (minor) and vitamin D compounds (Sabine 1977).

Sterols are typically eliminated via feces, urine, milk, and from the skin surface. Skin surface lipids contain 2% to 20% total sterols (Sabine 1977). Ling and Jones (1995) reported that phytosterol elimination via the biliary route appeared to be more rapid than that of cholesterol. The endogenous phytosterol pool size was low compared to cholesterol, due to poor intestinal absorption and faster excretion. The excretion rate of sitosterol from bile was 10 times greater than that of cholesterol (Gould 1955; American Cyanamid Co. 1975). Nearly complete recovery of administered phytosterols in mammals was made from the feces (Lange 1950; General Mills, Inc. 1979). Unabsorbed sterols (unspecified) were degraded in the intestinal tract to varying degrees, depending on the species: 5% in rats, 25% in humans, and 65% in monkeys (species not given) and baboons (Sabine 1977).

Effects of Phytosterols on Cholesterol Absorption and Metabolism

The plant sterols are effective inhibitors of cholesterol absorption in the small intestine, producing a hypocholesterolemic effect when the sterols are simultaneously ingested (Sabine 1977; Heinemann et al. 1991; Tvrzická et al. 1991; Heinemann, Axtmann, and von Bergmann 1993) in rabbits, chickens, rats, and humans (Peterson 1951; Laraki et al. 1993; Ling and Jones 1995). Phytosterols also interfere with the absorption of structurally different, unsaturated plant sterols. Plant sterol-induced decreases of sterol absorption may be directly related to the absorption efficiency of sterols (Vanhanen and Miettinen 1992).

Heinemann et al. (1991) reported that 20% to 70% of the 750 to 3000 mg/day total cholesterol (dietary and biliary) that entered the intestinal tract was absorbed after solubilization in mixed micelles containing bile salts, mono- and diglycerides, fatty acids, and lysolecithin. Simultaneous high-dose infusion of cholesterol and sitosterol decreased the overall absorption of cholesterol by 25% to 65% in an intestinal perfusion study using nine subjects. The reduction in cholesterol absorption was due to competition with cholesterol uptake in the micelles.

In a second intestinal perfusion study using 10 subjects, Heinemann, Axtmann, and von Bergmann (1993) reported that an inverse relationship existed between absorbability of plant sterols and their inhibition of cholesterol absorption. Phytosterol absorbability was reduced by hydrogenization of the 5a nucleus double bond or by an increase in the side chain length. Sterol absorption rates were 33% for cholesterol, 4.2% for sitosterol, 4.8% for stigmasterol, and 12.5% for campesterol. A positive correlation between the absorption rate of cholesterol and campesterol was established; a negative correlation was reported between the ratio of sitosterol to cholesterol and the mass of cholesterol absorption. Generally, the sterol absorption rate depended on micellar solubility. Cholesterol solubility was approximately three times greater than that of sitosterol. In addition, the binding of sitosterol to trihydroxy bile salt micelles was energetically favored over the binding of cholesterol. Hydrogenization of the delta-5-nucleus double bond caused a moderate enhancement of sterol hydrophobicity. This suggested that hydrophobic plant sterols with a high affinity and low capacity for micellar binding could have effectively displaced cholesterol from micellar binding.

When cultured in the presence of plasma lipoproteins, cells acquired cholesterol through receptor-mediated endocytosis of low-density lipoprotein (LDL). LDL-derived cholesterol esters in lysosomes were hydrolyzed, freeing unesterified cholesterol. Free cholesterol crossed the lysosomal membrane and was transported to other intracellular organelles. The rate of esterification of the plant sterols could have been the factor limiting their absorption. Esterification of endocytosed phytosterols in the endoplasmic reticulum was extremely low. Campesterol esterification was 20% that of cholesterol, and both sitosterol and stigmasterol were not esterified appreciably. When added to cell cultures, sitosterol and stigmasterol did not appear to be transported to intracellular membranes and, therefore, could not substitute for cholesterol to support cell growth. Instead, endocytosed plant sterols accumulated in the phagolysosomes of the cells. Phytosterol accumulation was not a consequence of the cell's inability to esterify sterols in the endoplasmic reticulum as cholesterol did not accumulate when its esterification was blocked. The side chain structure of sterols was critical for the efflux of sterols from lysosomes. Plant sterols were distinguished from cholesterol at the level of the intestinal mucosal cell, but the mechanisms have yet to be determined. The observations made in this study suggested that cultured macrophages were able to differentiate sterols that differed only by a methyl or ethyl group at the C24 position at their lysosomal compartment (Sato et al. 1995).

The conversion of sterols to bile acids is inefficient; phytosterols and other sterols can inhibit the synthesis of bile acids from cholesterol. Unmetabolized sterols secreted into bile were generally less soluble than cholesterol and can precipitate out if bile salt concentrations were reduced (Clayton et al. 1993).

Miscellaneous Phytosterol Effects

β-Sitosterol isolated from piper betle leaves (concentration not given) inhibited human platelet aggregation induced by arachidonic acid, platelet-activating factor, and ADP (Saeed et al. 1993).

A concentration of 25 μg/ml of phytosterols slightly decreased beat rates of fetal rat heart cells whereas similar additions of cholesterol increased beat rates (Hennessey 1992).

Phytosterols found in human tumors, particularly breast cancers, have osteolytic activity, and increase mobilization and excretion of bone calcium (Sabine 1977).

Exposure to 0.7 mmol/L (incorporated into liposomes) sitosterol for 72 hours caused contraction of human umbilical vein endothelial cells in vitro and an increased release of intracellular lactate dehydrogenase. At 96 hours, partial detachment from the substrate was observed. In addition, 0.35 mmol/L of sitosterol caused pertubation of the endothelial cells at 96 hours (Boberg, Pettersen, and Prydz 1991).

Sitosterol can have potent anti-inflammatory, antibacterial, and antifungal activities (Padmaja, Thankamany, and Hisham 1993). A β-sitosterol isolate had antiatherogenic effects through inhibition of platelet aggregation (Pollak 1985).

Results of an in vitro study by Chiang et al. (1991) indicated that β-sitosterol at a concentration of 100 μg/ml (5% in DMSO and saline) was cytotoxic against seven cancer cell lines: Colo-205 (colon), Hep-2 (laryngeal epidermoid), HeLa (uterine cervix), KB (nasopharynx), H1477 (melanoma), HA22T (hepatoma), and GBM8401/TSGH (glioma).

ANIMAL TOXICOLOGY

Acute Toxicity

Young, male Sprague-Dawley rats were given 10 g/kg doses (50% in feed) of PEG-5, -10, -16, or -25 Soy Sterol. No adverse

effects were reported and the acute oral LD_{50} for each compound was >10 g/kg (Warf Institute, Inc. 1974; Henkel Corp. 1995).

A liquid eyeliner containing 2% PEG-5 Soy Sterol was evaluated for dermal toxicity using 10 New Zealand white rabbits (North American Science Associates, Inc. 1987a). The rabbits each weighed between 2.5 and 3.1 kg. The test material (2 g/kg) was applied to the clipped upper back of each rabbit, at both intact and abraded skin sites. After application, the trunk of each rabbit was wrapped with polyethylene plastic that was taped in place, thus forming a reservoir over the test site. The rabbits were then fitted with collars and returned to their respective cages. The wrappings were removed after 24 hours, and the collars at test termination. The rabbits were observed for signs of toxicity immediately after treatment, at 4 hours, and daily for 14 days. Dermal reactions were scored daily for erythema and edema. On day 14, the skin sites were washed with tap water to remove any remaining residue of the test material. The rabbits were killed for necropsy. No animals died prior to test termination. Three of 10 rabbits had transient diarrhea, and one rabbit "appeared thin" during the last 6 days of the study. Dermal observations were slight redness (9/10), swelling (2/10) that diminished by day 6, and apparent pustules at the test site (1/10). Four rabbits lost between 0.4 and 0.6 kg of weight. No macroscopic changes of the viscera were observed at necropsy. The acute dermal LD_{50} was >2 g/kg, and the eyeliner was considered dermally nontoxic for the rabbit.

Polyethylene Glycol

Toxicity studies using rats, rabbits, and dogs indicate that PEGs have low oral and dermal toxicity (Andersen 1993). In general, the larger molecular weight PEGs appear to be less toxic than the smaller molecular weight PEGs in oral studies. Acute oral LD50 values for PEGs in rabbits were 17.3 g/kg (100% PEG-6) and 76 g/kg (100% PEG-75). In acute dermal toxicity studies, no deaths were reported in groups of rabbits dosed with undiluted PEG-6 (20 ml/kg) or 40% PEG-20M (20 ml/kg).

Short-Term Toxicity

Polyethylene Glycol

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

Phytosterols

In a study by Laraki et al. (1993), male adult Wistar rats weighing 215 ± 12 g were randomly assigned to eight dietary groups with 12 rats/group. Rats of each group were fed 22 g/day basal diet with or without supplementation by cholesterol or maize phytosterols (72.5% β-sitosterol, 20.5% campesterol, and 7% stigmasterol) for 3 weeks (Table 3). The basal diet contained 16% casein, 68% cornstarch, 8% butter, 4% cellulose, 3% mineral mix, and 1% vitamin mix. Water was available ad libitum. Feed consumption did not differ between treatment groups; mean consumption was 21.4 ± 0.1 g/day. The rats were killed and the livers sampled postmortem, washed, and frozen. Enzymatic activities of acetyl-CoA carboxylase, malic enzyme, and glucose-6-phosphate dehydrogenase were determined on microsomes purified from 1 g liver homogenized in 10 ml 0.25 M sucrose. Hepatic lipid and fatty acid compositions were determined.

TABLE 3
Diet composition (Laraki et al. 1993)

Group	1	2	3	4	5	6	7	8
Cholesterol (mg/day)	12	12	12	12	24	24	24	24
Phytosterol (mg/day)	—	12	24	48	—	12	24	48
Phytosterol:cholesterol ratio	0	1	2	4	0	1	2	4

The high cholesterol diet induced a significant increase in body weight (not specified) that was reduced by phytosterol supplementation. Rats of Group 2 (cholesterol and phytosterols, 12 mg/day each) had significantly enhanced liver weights and rats of Group 8 (24 mg/day cholesterol and 96 mg/day phytosterols) had reduced liver weights compared to the other groups. The mean liver weights were 11.1 ± 0.3 g for Group 2, 9.7 ± 1.1 g for Group 8, and 10.6 ± 0.2 g for the remaining groups.

No changes in enzyme activities were induced in rats fed the lower cholesterol dose (Groups 1–4), except in rats of Group 4, which had increased acetyl-CoA carboxylase activity compared to rats of Group 1. The higher cholesterol dose increased enzyme activities, but not significantly, in rats of Group 5, which did not receive phytosterols. In contrast, rats of Groups 6 to 8 had reduced acetyl-CoA carboxylase and malic enzyme activities. Rats given the high cholesterol dose had significantly decreased glucose-6-phosphate dehydrogenase activities compared to rats in the corresponding low-dose groups (i.e., the same phytosterol to cholesterol ratio).

For both cholesterol doses, increased hepatic phytosterol concentrations were detected after feeding of phytosterols. Stigmasterol was not detected in the liver, however. When phytosterols were not administered, the high cholesterol dose induced increases in hepatic cholesterol (Group 5 vs. Group 1). Hepatic cholesterol concentrations were reduced for all groups given phytosterols. The most efficient reductions occurred in groups with the highest phytosterol to cholesterol ratio. The cholesterol concentration was reduced by 7.6% in rats of Group 4 (low dose); a 32.4% decrease was observed in rats of Group 8 (high dose). Despite the difference in dietary cholesterol overload,

rats of both Groups 4 and 8 had the same hepatic cholesterol concentration.

Rats of Group 5 had a 2.5-fold increase in hepatic total fatty acids. This concentration reverted to normal after feeding of phytosterols, whatever the ratio of phytosterols to cholesterol. Fatty acid composition was not significantly modified.

The variations in malic enzyme and acetyl-CoA carboxylase activities were associated with variations of hepatic fatty acid content. The enhanced acetyl-CoA carboxylase activity of Group 4 rats was associated with an increase in the plasma triglyceride concentration. At the high cholesterol dose, feeding of phytosterols decreased the activities of malic enzyme and acetyl-CoA carboxylase to exert a hypotriglyceridemic effect. The observed hepatic fatty acid content decrease reflected a decrease in hepatic lipid content. In addition, the researchers concluded, phytosterols acted on rate-limiting enzymes of sterol metabolism and inhibited cholesterol digestive absorption. In the diet, the phytosterols decreased plasma and hepatic cholesterol when the phytosterol:cholesterol ratio was at least 2 (Laraki et al. 1993).

Wistar albino rats (10 per sex per group) were given subcutaneous injections of 250, 500, or 1000 μg/100 g/day β-sitosterol in olive oil for 60 days. No mortality was observed throughout the study. Treated rats given the lower doses had no gross or microscopic lesions of either the liver or kidneys. Mild fibroblastic proliferation around the hepatic lobules and microscopic lesions of the kidney were observed in animals given the high dose. These lesions were of very mild degree and had only a few heterophilic cell infiltrations in the medullary tract. All clinical biochemical parameters (hemoglobin, blood glucose, serum protein, serum bilirubin, glutamic-pyruvic transaminase [GPT] and glutamic-oxaloacetic transaminase [GOT]) were in the normal range with the exceptions of serum cholesterol and serum protein. Serum cholesterol was markedly depleted in a dose-dependent manner in both sexes. Serum protein was markedly reduced in the rats treated with 1000 μg/100 g/day of β-sitosterol (Malini and Vanithakumari 1990).

Subchronic Toxicity

Polyethylene Glycol

In subchronic, 90-day oral toxicity studies involving groups of albino rats, the largest (PEG-20M) and smallest (PEG-6) molecular weight PEGs tested did not induce toxicity or death when administered daily at concentrations of 4% or less; PEG-20M was administered in the diet and PEG-6 in drinking water. In a dermal toxicity study, no evidence of toxicity was observed in a group of rabbits that received daily applications of PEG-6 5 days per week (2 ml/kg/day) for 18 weeks (Andersen 1993).

Chronic Toxicity

Polyethylene Glycol

Toxic effects also were not observed in groups of dogs that received PEG-8, PEG-32, and PEG-75 at concentrations of 2% in the diet for 1 year (Andersen 1993).

Phytosterols

Thirteen dogs fed a basic diet supplemented with 0.5 to 1.0 g/kg/day of β-sitosterol had no gross or microscopic changes after 8 to 22 months of treatment. Weight gains and clinical parameters did not differ from controls (General Mills, Inc. 1979).

No adverse effects or gross or microscopic abnormalities were observed in six New Zealand white rabbits of both sexes that were given feed containing 3% cottonseed sterols and 4% soy sterols for 70 to 212 days (General Mills, Inc. 1979).

Dermal Irritation and Sensitization

Undiluted PEG-5, -10, -16, and -25 Soy Sterol were administered to the clipped back and flanks of albino rabbits (intact and abraded skin), six per group. The treated areas were covered with gauze patches, which were secured with tape. The patches were removed 24 hours later, and the treated sites were scored at that time and at 72 hours. The primary irritation index (PII) score was 0.50 for PEG-10 Soy Sterol and zero for PEG-5, -16, and -25 Soy Sterol (Warf Institute, Inc. 1974; Henkel Corp. 1995).

PEG-5 Soy Sterol at an induction and challenge concentration of 100% was a nonsensitizer in a study using Pirbright white guinea pigs (number of animals not stated) (Henkel Corp. 1995).

The primary skin irritancy of a liquid eyeliner containing 2% PEG-5 Soy Sterol was evaluated using six New Zealand white rabbits (North American Science Associates, Inc. 1987b). A 0.5-ml volume of the test sample was applied under a double gauze layer to both intact and abraded skin sites on 1 inch × 1 inch areas of the back. The patches were covered with nonreactive tape and the entire test site was wrapped with a binder. The binders and patches were removed after 24 hours, and the skin sites were rinsed with tap water. The sites were evaluated at 24 and 72 hours after application using Draize criteria. The PII was 0.96 (barely perceptible), and the investigators concluded that the eyeliner was not a primary skin irritant.

Polyethylene Glycol

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

Ocular Irritation

Undiluted PEG-5, -10, -16, and -25 Soy Sterol were instilled into the conjunctival sac of New Zealand white rabbits, six per group. The PEGs Soy Sterol did not induce ocular toxicity in any of the rabbits treated, and the ocular irritation score for each was zero (Warf Institute, Inc. 1974; Henkel Corp. 1995).

A 0.1-ml volume of a liquid eyeliner containing 2% PEG-5 Soy Sterol was instilled into the conjunctival sac of six New Zealand white rabbits. Prior to instillation, the eyes were

treated with fluorescein stain, flushed with saline, and observed in a darkened room under UV light to detect or confirm preexisting corneal injury. Ocular reactions were evaluated using Draize and Federal Hazardous Substances Act (FHSA) scoring criteria at 24, 48, and 72 hours after instillation. Minimal conjunctival redness was observed in 1/6 test eyes, but was not considered significant. The investigators concluded that the liquid eyeliner was not an ocular irritant (North American Science Associates, Inc. 1987c).

Polyethylene Glycol

PEGs -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).

Reproductive and Developmental Effects

Ethylene Glycol and its Ethers

It is generally recognized that the PEG monomer, ethylene glycol, and certain of its monoalkyl ethers (e.g., methoxyethanol, a.k.a. ethylene glycol monomethyl ether) are reproductive and developmental toxins. The CIR Expert Panel undertook a separate, limited scope review of these compounds in order to assess the possibility that PEG-derived cosmetic ingredients could present similar concerns (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 Soy Sterol, there is no likelihood of methoxyethanol, ethoxyethanol, etc., being present as an impurity. Further, the PEGs Soy Sterol are ethers of soybean oil sterols such as stigmasterol, γ-sitosterol, and campesterol and, as such, are chemically different from alkyl ethers.

Polyethylene Glycol

No adverse reproductive effects occurred during subchronic (90 days) and chronic (2 years) oral toxicity studies of PEGs 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 PEGs 6-32 at doses up to 1.69 g/kg/day (Andersen 1993).

Phytosterols

In male Wistar rats, subcutaneous injections of 0.5 to 5 mg/kg/day β-sitosterol for 16, 32, or 48 days reduced sperm concentrations, testis weights, and accessory sex tissue weights in a time-dependent manner. Within 30 days of withdrawal from treatment, the weight of accessory sex tissues were restored to near-normal conditions. In the same study, half of the long-term–treated rats were mated with unexposed females. Mated females were laparotomized on day 10 of pregnancy and the number of implantation sites was determined. Rats given 5 mg/kg/day of β-sitosterol had reduced fertility. Treatment with 0.5 mg/kg/day did not reduce the fertility of male rats, although a small (significant) decrease in sperm concentration of the caput epididymis was observed after 48 days of sitosterol treatment. Sperm concentration was defined as the sperm count $\times 10^6$/ml epididymal plasma. The control sperm concentration was 500 ± 42, and the sperm concentration of rats given the phytosterol was 366 ± 28. This decrease persisted, even after withdrawal of treatment, and appeared to be due to a reduction in the rate of spermatogenesis. Rats of the control groups received 0.5 or 5 mg/kg/day olive oil (Malini and Vanithakumari 1991).

β-Sitosterol acted as an effective estrogenlike agonist in producing vaginal cornification and caused uterine weight gain in adult, ovariectomized Wistar rats. In a short-term study, subcutaneous administration of β-sitosterol (50 μg/kg/day to 5.0 mg/kg/day) resulted in significant dose-related increases in uterine glycogen concentration after 10 days. At the highest dose, the glycogen concentration increase was equivalent to that produced by 50 μg/kg/day of estradiol. When β-sitosterol was given in combination with estradiol, the estradiol-induced glycogen concentration was slightly enhanced. Progesterone partially suppressed the phytosterol-induced elevation of glycogen concentration after the hormone (20 mg/kg/day) was administered in combination with median and high doses of β-sitosterol (2.5 and 5.0 mg/kg/day). In addition, treatment with β-sitosterol (all doses) stimulated glucose-6-phosphate dehydrogenase, phosphohexose isomerase, and total lactate dehydrogenase activities (Malini and Vanithakumari 1992).

In a later study by the same researchers, β-sitosterol administered alone or in combination with estradiol for 10 days caused a marked increase in the uterine weight of ovariectomized animals. When β-sitosterol was coadministered with progesterone, a twofold increase in uterine weight was observed. Estradiol administration resulted in a threefold increase. β-Sitosterol (50–500 μg/100 g/day), given alone, caused a progressive dose-dependent increase in uterine weight, with maximal increments occurring at the mid and high doses (250 and 500 μg/100g/day). Other effects reported were increased uterine RNA, DNA, and protein concentrations. These effects varied with coadministration of estradiol or progesterone. Estrogen and β-sitosterol induced DNA synthesis in the uterine luminal epithelium. Progesterone by itself stimulated a very small increase in DNA concentration; when coadministered with β-sitosterol, progesterone inhibited the growth promoting effect of the phytosterol. Epithelial DNA concentration increased by two-, three-, and seven-fold for the low, mid-, and high doses, respectively; however, the increases were only significant at the two latter doses. The investigators concluded that the dose-dependent uterotrophic effect of β-sitosterol in ovariectomized rats and its synergism with estradiol could be due to the phytosterol's intrinsic estrogenic properties, and that the effects of β-sitosterol could be inhibited by progesterone (Malini and Vanithakumari 1993).

Sulfates of β-sitosterol act as abortifacients in female rats and Dutch-belted rabbits via estrogenic and spermicidal effects. β-sitosterol itself had antiestrogenic, antiprogestational, gonadotrophic, antigonadotrophic, and antiandrogenic effects (Malini and Vanithakumari 1990; Burck, Thakkar, and Zimmerman 1982; Ling and Jones 1995).

Genotoxicity

PEG-5 Soy Sterol at 8 to 5000 μg/plate (in Tween 80/bidest water) was not cytotoxic to *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, or TA1538. The test compound, with or without metabolic (S9) activation, did not induce reverse mutations in those bacterial strains (Henkel Corp. 1995).

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

Polyethylene Glycol

All of the carcinogenicity data available on the PEGs was specifically on PEG-8, which was used as a solvent control for a number of studies. 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—rats; 1 year of dosing—mice), or when injected into the gastric antrum of guinea pigs over a period of 6 months (Andersen 1993).

Antitumorigenic Effects of Phytosterols

Evidence is available to suggest that phytosterols inhibit the induction of tumors in animals (Ling and Jones 1995). Sitosterol had an inhibitory effect on the tumor-promoting activity of 12-O-tetradecanoylphorbol-13-acetate (TPA) in mouse skin following initiation by 7,12-dimethylbenz[*a*]anthracene (DMBA). DMBA was administered as a single topical application of 50 μg to the shaved backs of female, 7-week-old ICR mice (20 per group). One week after initiation, promotion was begun with the twice weekly applications of 2.5 μg TPA. Thirty to 40 minutes after each TPA treatment, 5 μmol/100 μl doses of sitosterol or the vehicle, acetone-DMSO (9:1, 100 μl) were given. The backs of the treated mice were shaved weekly throughout the 18-week experiment. The first tumor in mice given TPA and DMSO without sitosterol was observed at week 5; all mice in this treatment group had skin tumors by week 10 (average = 21.1 tumors per mouse at week 18). In the group given sitosterol, 80% had skin tumors (average = 11.2 tumors per mouse at week 18). The percent reduction in the average number of tumors at week 18 was 40% in mice given TPA and DMSO plus sitosterol (Yasukawa et al. 1991).

Epidermal ornithine decarboxylase (ODC) activity induction is a characteristic biochemical alteration elicited by TPA, and can be representative of the effects of phorbol esters with strong tumor-promoting activity. Sitosterol applied topically 30 minutes prior to treatment with TPA inhibited TPA-induced ODC activity in five female ICR mice. In this study, 5 μmol of sitosterol was dissolved in 200 μl chloroform and applied to the shaved area of the skin. TPA (5 μg) was dissolved in acetone. Four hours after TPA administration, the mice were killed by cervical dislocation. The epidermis of the treated skin was separated by brief heat treatment, and ODC activity was determined in the soluble epidermal supernatant by measuring the release of $^{14}CO_2$ from [1-^{14}C]-ornithine. The results were expressed as nmol CO_2/30 min/mg protein. ODC activity was inhibited by 65% compared to the vehicle control (control, 2.8 ± 0.23; sitosterol, 1.3 ± 0.18). The researchers also reported that dermal inflammation caused by a single topical application of 1 μg TPA in mice was slightly reduced by sitosterol and stigmasterol (Yasukawa et al. 1991).

Male Fischer CD rats coadministered the direct-acting carcinogen *N*-methyl-*N*-nitrosourea and β-sitosterol had significantly fewer colon tumors (benign or benign and malignant) compared to rats given the carcinogen alone. The phytosterol was 95% pure, with 4% campesterol and 1% stigmasterol, and was given at a concentration of 0.2% in feed. The carcinogen (0.5 ml, in 0.9% saline; 2 mg/dose/rat) was administered by cannulation into the colon on days 1, 4, 7, and 10. Control rats (10 per group) received either control chow and intracolonic saline or control chow plus β-sitosterol and saline. No deaths occurred during the 28-week experiment, and no adverse effects were observed after feeding of the phytosterol. No tumors were detected in rats of either control group. Of rats given the carcinogen alone, 54% had tumors (1.1 tumor/rat and 2.1 tumors/tumor-bearing rat), and 78 tumors were detected. Thirty-three percent of rats that received the sterol-supplemented diet had tumors (0.44 tumor/rat and 1.3 tumor/tumor-bearing rat), and 21 tumors were found. Most lesions were adenomatous polyps. The incidence of malignant colonic neoplasms increased in rats given the phytosterol, however 7 of 48 mice (15%) had invasive carcinomas in the sterol plus carcinogen group compared to 5 of 71 mice (7%) in the carcinogen alone group (Raicht et al. 1980).

CELL PROLIFERATION EFFECTS OF PHYTOSTEROLS

Dietary addition of phytosterols (60% β-sitosterol, 30% campesterol, 5% stigmasterol) had a dose-related effect on colonic mucosal cell proliferation in female C57B1/6J mice. Six mice per group received 0.1% cholic acid with or without dietary supplementation of 0.1%, 0.3%, or 1.0% phytosterols. The mice were killed after 2 weeks, after being injected with

colchicine and [^{3}H]thymidine to determine the number of cells in the colonic crypts undergoing metaphase and DNA synthesis, respectively. Subsequently, the rate of colonic cell proliferation was determined. Dietary cholic acid significantly increased colonic epithelial cell proliferation and the highest labeled cell position by 92% and 35%, respectively. The mitotic index was 119%. A 1% concentration of dietary phytosterol did not significantly affect cell proliferation compared to the control group, but the mitotic index indicated a significant decrease in the number of cells in mitosis after 0.1% phytosterol was given (Janezic and Rao 1992).

Phytosterol can decrease epithelial cell proliferation by suppressing the bacterial metabolism of cholesterol and/or secretory bile acids in the colon and by increasing excretion of cholesterol. Dietary phytosterols appear to inhibit colonic cancer development prior to adenoma formation (Ling and Jones 1995).

At concentrations of 10^{-3} to 10^{-6} M, stigmasterol and campesterol from stinging nettle root extracts inhibited the membrane Na^+/K^+-ATPase activity of benign prostatic hyperplasia by 23% to 67%. This inhibition could have subsequently suppressed prostate cell metabolism and growth (Hirano, Homma, and Oka 1994).

A mixture of β-sitosterol, stigmasterol, campesterol, and ducitol isolated from *Gymnosporia trilocularis* was cytotoxic (ED_{50} = >20 mcg/ml) for a human cell line of an epidermoid carcinoma of the nasopharynx (KB) test system (Ling et al. 1981).

Dietary β-sitosterol at a concentration of 0.2% decreased the rate of colonic epithelial cell proliferation and compressed the crypt's proliferative compartment, thus suppressing expression of the altered genome, in Fischer rats induced with *N*-methyl-*N*-nitrosourea. Six rats were treated with the phytosterol alone, three were given the carcinogen (by cannulation), and five were given both the sterol and the carcinogen. Six rats served as control and were given stock feed only. Cell proliferation was reduced in rats given *N*-methyl-*N*-nitrosourea plus β-sitosterol as early as 3 days after initiation of feeding, as compared to rats given the carcinogen alone, and at 28 weeks, when the rats were killed. The mean number of radioactive cells per crypt column was 3.3 for rats coadministered β-sitosterol and 5.4 in rats given *N*-methyl-*N*-nitrosourea (Deschner, Cohen, and Raicht 1982).

CLINICAL ASSESSMENT OF SAFETY

Dermal Irritation and Sensitization

Twelve volunteers, aged 27 to 54 years, were patch tested with a mascara containing 2% PEG-5 Soy Sterol in a modified Draize repeat insult patch test (RIPT) to determine irritation potential (Biosearch Incorporated 1992a). On a Friday, 0.75 inch × 0.75 inch gauze pads (evenly covered with the test material) were applied to skin sites on the back. The occlusive patches were removed at 24 hours. The procedure was repeated on the following Monday, Tuesday, and Wednesday. The skin sites were evaluated 48 hours after the final application. All subjects completed the study. No evidence of skin irritation was observed.

A different modified Draize RIPT was performed using the same formulation and 84 subjects, 75 of whom completed the study (Biosearch Incorporated 1992b). Approximately 0.01 g of the test material was applied to cover the gauze pad of an occlusive patch. The patch was applied to the back and removed after 24 hours. The procedure was repeated 3 alternate days/week until nine applications had been made. Fourteen days after the last induction patching, a challenge patch was applied to an adjacent skin site (untreated). The patch was removed at 24 hours, and the site was examined at 24, 48, and 72 hours. No evidence of irritation or sensitization was observed.

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).

Photosensitization

A Schwartz-Peck prophetic patch test was used to determine primary skin irritation of a liquid eyeliner containing 2% PEG-5 Soy Sterol (Biosearch Incorporated 1987). A group of 101 female subjects, aged 16 to 63 years, was used in this study. Approximately 0.2 g of the test formulation was applied to the upper back using a 0.75-inch diameter Lintine disc. The patch was then covered with cloth tape and left on the skin for 48 hours. Simultaneously, ~0.1 g of the material was applied to the volar forearm as an open patch. The exposed skin sites were examined 48 hours after application. Fourteen days later, this procedure was repeated. In addition, the site on the back was exposed to UV light (wave length = 3650 Å) at a distance of 12 inches for 1 minute. After the second insult application, the closed patch site was irradiated. The application sites were reexamined after 48 hours to determine if photosensitization had occurred. No signs of primary skin irritation or photosensitization were observed during this study.

A Draize-Shelanski RIPT was performed using a liquid foundation containing 0.75% PEG-5 Soy Sterol and 1.5% PEG-25 Soy Sterol (Biosearch Incorporated 1991). Eighty-eight subjects (16–55 years old) were enrolled in the study, and 77 completed it. A 0.2-ml volume of the foundation was applied (on a 0.75-inch diameter Lintine disc) to a skin site on the back. The patch was covered with cloth tape. At the same time, 0.2 ml of

the test material was applied to the volar forearm as an open patch. At 48 hours, the patches were removed and the skin sites were examined. This procedure was repeated three times a week for $3^1/_2$ weeks. (The application site was divided into quadrants such that three were used for induction patching and one for challenge. The first quadrant received the first, fourth, seventh, and tenth applications; the second received the second, fifth, and eighth applications; and the third received the third, sixth, and ninth applications.) Fourteen days later, identical open and closed patches were applied and evaluated at 48 hours. In addition, the closed patch was irradiated for 1 minute using a UV light source (3650 Å) after the first, fourth, seventh, tenth, and eleventh applications. The subjects were examined 48 hours later to determine if photosensitization had occurred. During this study, no signs of primary irritation, sensitization, or photosensitization was observed.

Ten female volunteers (25–47 years old) were used in a phototoxicity study of a mascara containing 2% PEG-5 Soy Sterol (Biosearch Incorporated 1992c). A filtered (150 W) UV solar simulator was used to provide a continuous output in the UVB and UVA region (290–400 nm). For exposure to UVA only, a filter was used to eliminate UVB wavelengths (290–320 nm). The shutter of the solar simulator was controlled to be closed upon completion of 14 J/cm^2. Prior to the start of the study, each subject's minimum erythemal dose (MED) was determined using skin sites on the back. For the study, the test material (20 μl) was applied to two sites on the back (diameter = 1.5 cm). The third site was untreated and served as an irradiation control site. One treated site and the control site were irradiated 30 to 60 minutes after application with 0.5 MED of UVB and UVA, and then with 14 J/cm^2 of UVA. The sites were evaluated at 24, 48, and 72 hours. No reactions were observed at either of the treated sites.

The photosensitization potential of the same formulation was determined in a study using 30 subjects, aged 16 to 50 years (Biosearch Incorporated 1992d). Twenty-nine subjects completed the study. In this study, the shutter closed when 4 J/cm^2 were delivered. Approximately 0.1 g of the mascara formulation was applied to the gauze pad of an occlusive patch, which was then affixed to the skin of the back. The patches were applied on Mondays and Wednesdays of 3 consecutive weeks, and remained in place for 24 hours each time. After patch removal, the sites were exposed to 2.0 MEDs of UVB irradiation and 4 J/cm^2 of UVA irradiation. On day 18 after the last induction exposure, the subjects were patched at two separate adjacent untreated sites. The patches were removed at 24 hours, and the sites were examined. One site was exposed to 0.5 MED of UVB and 4 J/cm^2 of UVA. One untreated site was similarly irradiated. The challenge sites were evaluated at 24, 48, and 72 h after irradiation. No evidence of photosensitization was observed.

Comedogenicity

Sixty-two female subjects were used to evaluate the comedogenicity of sun protection factor (SPF) 15 liquid foundation containing 0.75% PEG-5 Soy Sterol and 1.5% PEG-25 Soy Sterol (The Educational & Research Foundation, Inc. 1991a). Fifty-nine of the panelists completed the study. The subjects were between the ages of 14 and 40. Prior to the study applications, a dermatologist examined each subject for irritation and evaluated all noninflammatory lesions (closed and open comedones) and inflammatory lesions (papules, pustules, and nodules). Thirty subjects had mild acne (>10 noninflammatory and inflammatory lesions), and 32 had no acne (<10 lesions). The subjects used the foundation twice a day for 49 consecutive days. On days 7 and 28, a clinical laboratory technician examined the panelists for tolerance of the product and participant cooperation with testing instructions. The dermatologist examined the subjects at study termination. Several subjects reported stinging and burning sensations, but erythema and scaling were not observed by the dermatologist or technician. The total scores for subjects with acne decreased from 557 to 397 during the study, and the scores for nonacne subjects remained the same (56 to 55). The foundation was "well tolerated" by the panel, and did not cause comedogenicity in either the acne-prone or non-acne groups.

In a second (45-day) study, SPF 15 liquid foundation (0.75% PEG-5 Soy Sterol, 1.5% PEG-25 Soy Sterol) was evaluated using 56 female panelists, 52 of whom completed the study (The Educational & Research Foundation, Inc. 1991b). Twenty-seven of the panelists had mild acne and 29 had no acne. The subjects used the foundation twice a day (minimum) for 45 consecutive days. On days 28 and 45, the dermatologist examined all subjects for comedones, papules, pustules, and nodules, and a clinical laboratory technician examined them to determine tolerance to the product and participant cooperation with testing instructions. Several of the panelests reported itching during the course of the study. One of the subjects had itching and a face rash at the final visit; at subsequent patch testing, no signs of allergenicity were observed. All subjects had reductions in acne scores, and with the exception of the subject with the rash, none had signs of irritation. No allergic reactions were observed.

Ocular Irritation

A mascara containing 2% PEG-5 Soy Sterol was evaluated for ocular irritancy using 60 female subjects (North Cliff Consultants 1992). Each subject was examined by an ophthalmologist prior to the study. Each subject used the mascara twice daily for 4 weeks, and was examined weekly by a trained clinical technician and the ophthalmologist. Four subjects did not complete the study. Several subjects reported sensations of irritation, but this could not be correlated with the evaluations made by the technician. Eighteen subjects, 9 of whom were contact lens wearers, reported itching and/or irritation at least once during the study. Clinical signs of irritation (slight erythema) were observed around the eyes of 23 subjects (11 contact wearers).

The cutaneous effects of an eyeliner containing 2% PEG-5 Soy Sterol were evaluated during a clinical usage study using 60 female subjects between the ages of 18 and 35 (Biosearch Incorporated 1988). Of the subjects, 26 wore contact lenses.

Two contact lens wearers and one other subject did not complete the study. The subjects were examined by a dermatologist and ophthalmologist prior to the start of the study. The subjects applied the eyeliner twice daily for 28 consecutive days. They were examined by a clinical technician weekly, and were reexamined by a dermatologist and an ophthalmologist at the conclusion of the study. Of the non-contact wearers, one subject had a moderate amount of mucoid discharge with blue particles on both eyes, but was asymptomatic. Two subjects complained of burning sensations. Five subjects had inert "chunks" of the product in their conjunctiva which were not noticed by the subjects. None of the non–contact lens wearers had visual findings indicative of ocular irritation at the final examination. Several contact lens wearers had signs of ocular irritation, but irritation is not uncommon among individuals wearing contact lenses. Six subjects had changes from their baseline examinations: five subjects had changes that were "exclusively related to their contact lenses," and one had smeary lenses to which adhered numerous fine particles.

Clinical Effects of Phytosterols

The phytosterols are often used to lower cholesterol by blocking its absorption (Pollak 1953; Shipley et al. 1958). Patients with hypercholesteremia given daily doses of sitosterols for periods exceeding 4 years had no signs of adverse effects (Shipley et al. 1958). Upon cessation of phytosterol intake, hemocholesterols returned to the original concentration (Pollak 1953).

Subjects were continuously administered sitosterols in the diet for periods exceeding 4 years, during which the total amount of sitosterol consumed was greater than 50% of the patient's body weight. Kidney and liver function, blood and urine composition, electrocardiogram, and gall bladder visualization were not different from controls. Treatment with sitosterol also did not contribute to the formation or progression of vascular lesions (General Mills, Inc. 1979).

Phytosterolemia is the impaired lipid metabolism characterized by the accumulation of free and esterified plant sterols and cholesterol in blood and tissues (Tvrzická et al. 1991) due to excessive intestinal absorption of sterols. Patients with hereditary phytosterolemia develop xanthomata, thrombocytopenia, hemolytic anemia, and premature atherosclerosis (Clayton et al. 1993).

SUMMARY

PEG-5, -10, -16, -25, -30, and -40 Soy Sterol are PEG derivatives of soybean oil sterols. They function as nonionic surfactants, and as emulsifying, skin conditioning, cleansing, and solubilizing agents. The PEGs Soy Sterol are also used as appearance and consistency modifiers, emollients, viscosity control agents, and pigment dispersion agents. In 1996, the PEG-5, -10, -16, -25, and -40 Soy Sterol were used in 41, 35, 15, 5, and 1 cosmetic formulations, respectively, and PEG-30 Soy Sterol was not used. The current concentrations of use were not available, but in 1984, PEG-5 and -10 Soy Sterol were used at concentrations up to 5%, and PEG-16 and -25 Soy Sterol were used at concentrations up to 1%. Recent data supplied by industry indicated that PEG-5 Soy Sterol was used at 2% in mascara and eyeliner. A liquid foundation contained 0.75% PEG-5 Soy Sterol and 1.5% PEG-25 Soy Sterol.

Few data were available on the PEGs Soy Sterol. Relevant data on the PEGs and Soy Sterols were included with the assumption that they were pertinent to the safety assessment of the PEGs Soy Sterol.

The PEGs Soy Sterol are produced by the reaction of the soy sterol hydroxyl with n moles of ethylene oxide. The soy sterols (phytosterols) include γ-sitosterol, campesterol, stigmasterol, and possibly β-sitosterol.

PEGs can contain small amounts of monomer and dimers, as well as peroxides formed during autoxidation. Ethoxylated surfactants can contain 1,4-dioxane, which is removed during purification from cosmetic ingredients prior to blending into cosmetic formulations. Refined plant sterols consist of approximately 88% total soy sterol, of which 56% is γ-sitosterol, 28% is campesterol, and 4% is stigmasterol. Impurities of the phytosterols are 4% to 6% sterol hydrocarbons and cholesterol, and 4% to 6% triterpene alcohols, keto-steroids, and other steroid-like substances.

The phytosterols are structurally similar to cholesterol. Approximately 0.25 to 0.5 g of plant sterols are consumed in a typical daily diet (20–25% of total dietary sterols). The phytosterols affect plant membrane structure and water permeability, and are commonly found in animal cell membranes after dietary intake. Phytosterols are less water soluble than cholesterol, but sitosterol and campesterol order bilayer acyl chains more effectively than cholesterol and stigmasterol.

Gastrointestinal absorption of PEGs was dependent on the molecular weight of the compound. The greater the molecular weight, the less the absorption. No metabolism was observed during oral and intravenous studies, and the PEGs were rapidly eliminated unchanged in the urine and feces. PEGs were readily absorbed through damaged skin.

Once consumed, phytosterols only entered the body via intestinal absorption. The absorption rate for the plant sterols was usually less than 5% of dietary concentrations in humans. Saturated sterols were virtually not absorbed, and approximately 95% of dietary phytosterols entered the colon. During subcutaneous injection studies using dogs, the phytosterols were not esterified or metabolized, and were treated like inert, foreign materials. Cholesterol, when similarly injected, was readily esterified. In other studies, sitosterol did not accumulate in tissues.

Freshly absorbed sterols were transported into plasma. The serum concentrations of phytosterols after dietary supplementation increased to 10% of total serum sterols. During metabolism and excretion, the sterol rings generally remained intact, whereas double bonds, constituent groups, and side chains were often added, removed, or modified. The largest proportion of sterol (in humans, 20% of absorbed β-sitosterol) in the body was

converted to bile acids. Absorbed phytosterols not converted to normal bile acids were excreted as free sterol. β-Sitosterol can be metabolized to 5,6-epoxides in the liver, cortisol in the adrenal glands, and various steroid hormones in the testes. In general, phytosterols can act as plant hormone and hormone precursors, and can be metabolized to minor steroid hormones and vitamin D compounds.

Sterols were typically eliminated via feces, urine, milk, and from the skin surface. The excretion rate of sitosterol from bile was 10 times greater than that of cholesterol. Nearly complete recovery of administered phytosterols in mammals was made from the feces. Unabsorbed sterols were degraded in the intestinal tract to varying degrees, depending on the species: 5% in rats, 25% in humans, and 65% in monkeys and baboons.

Plant sterols inhibited absorption of cholesterol in the small intestine, and produced a hypocholesterolemic effect when the sterols were simultaneously ingested by rabbits, chickens, rats, and humans. Phytosterols also interfered with the absorption of other structurally different, unsaturated plant sterols. During human intestinal perfusion studies, the overall absorption of cholesterol decreased by 25% to 65%. An inverse relationship existed between absorbability of plant sterols and their inhibition of cholesterol absorption.

β-Sitosterol inhibited human platelet aggregation induced by arachidonic acid, platelet-activating factor, and ADP. A dose of 25 μg/ml phytosterols slightly decreased beat rates of fetal rat heart cells, whereas similar doses of cholesterol increased beat rates. Phytosterols in human neoplasms (particularly breast cancers) had osteolytic activity, and increased mobilization and excretion of bone calcium. Human umbilical vein endothelial cells contracted in vitro after being exposed to 0.7 mmol/L sitosterol for 72 hours, and an increased release of intracellular lactate dehydrogenase was observed. At 96 hours, the cells were partially detached from the substrate, and pertubation of the endothelial cells occurred after exposure to 0.35 mmol/L. Sitosterol had potent anti-inflammatory, antibacterial, and antifungal activities, and β-sitosterol had antiatherogenic effects through the inhibition of platelet aggregation. β-Sitosterol (100 μg/ml; 5% in DMSO and saline) was cytotoxic against seven cancer cell lines.

The acute oral LD_{50} in rats of PEG-5 to -25 Soy Sterol was >10 g/kg (50% in feed). The acute dermal LD_{50} of a liquid eyeliner containing 2% PEG-5 Soy Sterol was >2 g/kg. The acute oral LD_{50} values of the PEGs in rabbits were 17.3 g/kg and 76 g/kg for undiluted PEG-6 and PEG-75, respectively. No deaths were reported when groups of rabbits were treated topically with 20 ml/kg undiluted PEG-6 or 40% PEG-20M.

Transient, mild erythema was observed when rabbits were given daily topical applications of 0.8 g/kg/day PEG-20M for 30 days. Signs of systemic toxicity were observed only in rabbits that received repeated applications of an antimicrobial cream that contained 63% PEG-6, 5% PEG-20, and 32% PEG-75 to excised skin sites for seven days.

Wistar rats that received a basal diet supplemented with cholesterol and maize phytosterols (72.5% β-sitosterol, 20.5% campesterol, and 7% stigmasterol) had decreased hepatic cholesterol concentrations. Rats given the high dose of cholesterol and phytosterols had decreased malic enzyme and acetyl-CoA carboxylase activities, and had hypotriglyceridemia.

Wistar rats given subcutaneous injections of 250 to 500 μg/100 g β-sitosterol for 60 days had no gross or microscopic lesions of the liver or kidneys. Rats given 1000 μg/100 g had mild fibroblastic proliferation around the hepatic lobules and mild microscopic lesions of the kidney. Serum cholesterol was depleted in a dose-dependent manner, and serum protein was markedly reduced in rats of the high dose group.

PEG-20M and PEG-6 (≤4% in feed and drinking water, respectively) did not cause death or induce other evidence of toxicity in albino rats dosed daily for 90 days. Rabbits that received daily topical applications of PEG-6 5 days per week for 18 weeks had no signs of toxicity.

Dogs given PEG-8, PEG-32, and PEG-75 at concentrations of 2% in the diet for 1 year had no signs of toxicity. Dogs given feed containing 0.5 to 1.0 g/kg/day β-sitosterol had no gross or microscopic lesions after 8 to 22 months of treatment, and weight gain and clinical parameters did not differ from controls. Rabbits fed a diet containing 3% cottonseed sterols and 4% soy sterols for 70 to 212 days had neither clinical signs of toxicity nor gross and microscopic abnormalities.

PEG-5 to -25 Soy Sterol were not primary irritants when applied undiluted or up to 2% in formulation to intact and abraded skin of six rabbits per group. Undiluted PEG-5 Soy Sterol did not cause sensitization in a maximization study using Pirbright white guinea pigs. PEG-6 and -8 were not irritating to the skin of rabbits or guinea pigs, and PEG-75 was nonsensitizing to the skin of guinea pigs.

Undiluted PEG-5, -10, -16, and -25 Soy Sterol and 2% PEG-5 Soy Sterol in formulation did not induce ocular toxicity when instilled into the conjunctival sac of six New Zealand white rabbits. Undiluted PEG-6 and PEG-75 did not cause corneal injuries when instilled into the conjunctival sac of rabbits. A 35% solution of PEG-8 and PEG-32 (melted in a water bath) induced mild ocular irritation in rabbits.

No adverse reproductive effects occurred during subchronic (90 days) and chronic (2 years) oral studies of 0.062 to 1.69 g/kg/day PEG-6 to -32 and PEG-75. Although monoalkyl ethers of ethylene glycol are reproductive toxins and teratogenic agents, given the methods of manufacture of the PEGs Soy Sterols, there is no likelihood of methoxyethanol, ethoxyethanol, etc., being present as an impurity. Further, the PEGs Soy Sterols are ethers of soybean oil sterols, and as such, are chemically different from alkyl ethers. It is considered unlikely that the PEGs Soy Sterols would cause reproductive or developmental effects based on their structural characteristics.

Subcutaneous injections of 5 mg/kg/day of β-sitosterol for 16 to 48 days reduced sperm concentrations and fertility, and decreased testis and accessory sex tissue weights (time dependent) in male Wistar rats. Rats given 0.5 mg/kg/day had a significant decrease in sperm concentration of the caput epididymis after

48 days of treatment, but no reduction in fertility. The observed decreases in sperm concentration persisted after withdrawal of treatment, and appeared to be due to a reduction of the rate of spermatogenesis.

β-Sitosterol was an effective estrogenlike agonist in exerting vaginal cornification and caused uterine weight gain in adult, ovariectomized Wistar rats. Subcutaneous injections of the sterol caused dose-related increases in uterine glycogen concentration after 10 days. Progesterone treatment partially suppressed the phytosterol-induced elevation of glycogen concentration when administered in combination with the median and high phytosterol doses. β-Sitosterol also stimulated glucose-6-phosphate dehydrogenase, phosphohexose isomerase, and total lactate dehydrogenase activities. In a related study, uterine RNA, DNA, and protein concentrations were increased by treatment with β-sitosterol.

Sulfates of β-sitosterol acted as abortifacients in female rats and Dutch-belted rabbits via estrogenic and spermidicidal effects. β-Sitosterol itself had antiestrogenic, antiprogestational, gonadotrophic, antigonadotrophic, and antiandrogenic effects.

PEG-5 Soy Sterol was not cytotoxic or mutagenic to five strains of *S. typhimurium*, with or without S9 activation. PEG-8 (up to 1%) was negative in the Chinese hamster ovary cell mutation test and the sister chromatid exchange test. At 0.1%, the highest concentration tested, a statistically significant increase in radioactive thymidine incorporation into rat hepatocyte nuclei was noted in the unscheduled DNA synthesis assay. PEG-150 was not mutagenic in the mouse lymphoma forward mutation assay when tested at concentrations up to 150 g/L.

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

Sitosterol inhibited the tumor-promoting activity of TPA in the skin of female ICR mice after initiation with DMBA. The percent reduction in the average number of tumors at week 18 was 40% in mice given TPA, DMBA, and sitosterol. Sitosterol applied topically before treatment with TPA inhibited TPA-induced epidermal ODC activity; ODC induction can be representative of the effects of phorbol esters with strong tumor-promoting activity. Additionally, dermal inflammation caused by a single application of TPA was slightly inhibited by sitosterol and stigmasterol.

Male Fischer CD rats coadministered the direct-acting carcinogen *N*-methyl-*N*-nitrosourea (by cannulation) and β-sitosterol (95% pure, with 4% campesterol and 1% stigmasterol; 0.2% in feed) had significantly fewer colonic tumors (benign or benign and malignant) compared to rats given the carcinogen alone after 28 weeks. Of rats given the carcinogen alone, 54% had tumors. Of rats given both the carcinogen and sitosterol, 33% had tumors. The incidence of rats with malignant colonic neoplasms increased after coadministration of the phytosterols; 15% (7/48) had invasive carcinomas in the sterol plus carcinogen group compared to 7% (5/71) of rats given the carcinogen alone.

The phytosterols decreased epithelial cell proliferation of the colon in mice (0.1% in feed) and rats (0.2% in feed after induction with *N*-methyl-*N*-nitrosourea), and were cytotoxic for human epidermoid carcinoma of the nasopharynx (>20 mcg/ml).

PEG-5 Soy Sterol at concentrations up to 2% in formulation did not cause dermal or ocular irritation, dermal sensitization, or photosensitization in clinical studies. A formulation containing PEG-5 Soy Sterol and PEG-25 Soy Sterol (at concentrations of 0.75% and 1.5%, respectively) was tested for comedogenicity and sensitization; no increase in comedones and no signs of irritation or allergic reactions were observed. PEG-6 and PEG-8 were mild sensitizers during a clinical study, but later production lots of PEG-6 and PEG-75 did not cause sensitization. A product formulation containing 3% PEG-8 was a minimal to mild skin irritant. Burn patients that received PEG-based topical ointments had cases of systemic toxicity and contact dermatitis.

DISCUSSION

The CIR Expert Panel was concerned about the sensitization potential of the PEG-5, -10, -16, -25, -30, and -40 Soy Sterol 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 determined to be the causitive agent in both animal and human studies; no evidence of systemic toxicity or sensitization was found in studies with intact skin. The Expert Panel concluded that cosmetic formulations containing PEG should not, therefore, be used on damaged skin.

The Panel members stressed that the cosmetic industry should continue to use the necessary purification procedures to remove possible 1,4-dioxane and ethylene oxide impurities from the ingredients before blending them into cosmetic formulations.

The Expert Panel was also concerned about the possible presence of free phytosterols. β-Sitosterol, in particular, had antiestrogenic, antiprogestational, gonadotrophic, antigonadotrophic, and antiandrogenic effects in various animal studies. This phytosterol reduced the fertility of male rats by reducing the rate of spermatogenesis and had spermicidal effects.

Additional data, however, were considered necessary to assess the safety of the PEGs Soy Sterol. Section 1, paragraph (p) of the CIR Procedures states that "a lack of information about an ingredient shall not be sufficient to justify a determination of safety." In accordance with Section 30(j)(2)(A) of the Procedures, the Expert Panel informed the public of its decision that the data on PEG-5, -10, -16, -25, -30, and -40 Soy Sterol were not sufficient for determining whether the ingredients, under relevant conditions of use, were safe or unsafe. The Panel released an Insufficient Data Announcement on June 4, 1996, outlining the data needed to assess the safety of the PEG Soy Sterol compounds. No comments or data were received in response to the announcement. Additional data needed to make a safety assessment are: (1) impurities data; (2) current concentration of use; (3) genotoxicity in a mammalian system of PEG-5 Soy Sterol; if positive, then a 2-year dermal carcinogenicity study using

NTP methods is needed; (4) skin sensitization and irritation in humans at concentration of use; and (5) dermal absorption of PEG-5 Soy Sterol; if significantly absorbed, then both a 28-day dermal toxicity study and reproductive and developmental toxicity data may be needed (alternatively, data showing that dietary intake results in the release/availability of phytosterols would suffice).

Because these data needs were identified, additional data were received, including: acute dermal toxicity in rabbits, primary skin and ocular irritation in rabbits, and human clinical studies for skin irritation and sensitization, ocular irritation, comedogenicity, phototoxicity, photosensitization, and safety in-use. Although these studies address the Expert Panel's concern about the irritation and sensitization potential in humans of the PEGs Soy Sterol, they do not address the Panel's other concerns. The additional data needed include (1) impurities; (2) genotoxicity in a mammalian system; if positive, then a 2-year dermal carcinogenicity study using NTP methods is needed; and (3) dermal absorption of PEG-5 Soy Sterol; if significant absorption occurs, then a 28-day dermal toxicity study is needed. Depending on the results of the 28-day study, a reproductive and developmental toxicity study may be needed.[2]

CONCLUSION

The CIR Expert Panel concludes that the available data are insufficient to support the safety of PEG-5, -10, -16, -25, and -40 Soy Sterol for use in cosmetic products.

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[2]Although the CIR Expert Panel has specified a "28-day dermal toxicity study," there is concern that specifying a type of study may inhibit those who want to gather data using other study designs. The types of data the Panel is seeking include the gross pathology and histopathology in skin and other major organ systems, along with certain other toxicity parameters, associated with repeated exposures. Doing a 28-day dermal toxicity study would generate the needed data. But there are other approaches. For example, the Expert Panel would consider a dermal reproductive and developmental toxicity study in which gross pathology and histopathology data are gathered on the F_0 generation to be sufficient to meet the "28-day dermal toxicity and dermal developmental/reproductive data" requested in item 3, if done at or above current concentrations of use of the ingredient.

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