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

PEGs Soy Sterol are polyethylene glycol (PEG) derivatives of soybean oil sterols used in a variety of cosmetic formulations as surfactants and emulsifying agents, skin-conditioning agents, and cleansing and solubilizing agents. When the safety of these ingredients were first reviewed, the available data were insufficient to support safety. New data have since been received and the safety of these ingredients in cosmetics has been substantiated. Current concentration of use ranges from a low of 0.05% in makeup preparations to 2% in moisturizers and several other products. 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 campesterol, stigmasterol, and β-sitosterol. The distribution of sterols found in oils derived from common plants is similar, with β-sitosterol comprising a major component. Impurities include sterol hydrocarbons and cholesterol (4% to 6%) and triterpine alcohols, keto-steroids, and other steroid-like substances (4% to 6%). No pesticide residues were detected. PEGS: Because PEGs are an underlying structure in PEGs Soy Sterols, the previous assessment of PEGs was considered. 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. Also, 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. Plant Phytosterols: Intestinal absorption of ingested plant phytosterols is on the order of 5%, with 95% of the material entering the colon. Absorbed plant phytosterols are transported to the blood. Although there are some data suggesting that sulfates of β-sitosterol can act as abortifacients in rats and rabbits, other studies of well-characterized plant phytosterols and phytosterol esters demonstrated no effect in an estrogen-binding study, a recombinant yeast assay for estrogen or estrogen-like activity, or a juvenile rat uterotrophic assay for estrogen or estrogen-like activity. In a two-generation reproduction study using rats, plant phytosterol esters in the diet had no effect on any parameter of reproduction or fertility. Subcutaneous injections of β-sitosterol did reduce sperm concentrations and fertility in rats. Sitosterol inhibited tumor promoting activity of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in mice after initiation with 7,12-dimethylbenz[*a*]anthracene (DMBA), and reduced the tumors produced by *N*-methylnitrosourea in rats. Phytosterols were not genotoxic in several bacterial, mammalian, and in vitro assay systems. Phytosterols decreased epithelial cell proliferation in the colon of mice and rats, and were cytotoxic for human epidermoid carcinoma of the nasopharynx. PEGs Soy Sterols: The acute oral LD_{50} in rats of PEG-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 in rabbits. PEG-5–25 Soy Sterol was not a primary irritant 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. 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, it was recommended that PEGs Soy Sterol should not be used in cosmetic products applied to damaged skin. Although no dermal absorption data were available, oral studies demonstrate that phytosterols and phytosterol esters are not significantly absorbed and do not result in significant systemic exposure. Some small amounts did appear in the ovaries, however. This raises a concern about the potential presence of free phytosterols and β-Sitosterol, which could have antiestrogenic, antiprogestational, gonadotrophic, antigonadotrophic, and antiandrogenic effects in PEG sterols. These concerns are alleviated by the extensive data showing that well-defined phytosterols and phytosterol esters are not estrogenic and do not pose a hazard to reproduction. Likewise, the absence of impurities in plant phytosterols and phytosterol esters and extensive data demonstrating the absence of any genotoxicity in bacterial and mammalian systems mitigate against the possibility of any carcinogenic effect with those same well-characterized materials. The Cosmetic Ingredient Review (CIR) Expert Panel concluded that the PEGs Soy Sterol are safe as used 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 non-ionic surfactants and emulsifying agents in cosmetic formulations. The safety of these ingredients was first reviewed by the Cosmetic Ingredient Review (CIR) Expert Panel in a series of meetings leading to a conclusion in 1997 that the

Received 12 February 2004; accepted 4 June 2004.

[1]Reviewed by the Cosmetic Ingredient Review Expert Panel. This report was prepared by Eric Hooker, Scientific Analyst and Writer. Address correspondence to Eric Hooker, 1101 17th Street, NW, Suite 310, Washington, DC 20036, USA.

International Journal of Toxicology, 23(Suppl. 2):23–47, 2004

ISSN: 1091-5818 print / 1092-874X online
DOI: 10.1080/10915810490499046

available data were not sufficient to support the safety of this ingredient in cosmetic products (CIR 1997). One concern was the presence of free phytosterols in the soy sterol component of the PEGs Soy Sterol group of ingredients and the potential reproductive toxicity of β-sitosterol, in particular. The Expert Panel also sought impurities information and genotoxicity data.

New information has been provided that characterizes phytosterols and phytosterol fatty acid esters in general, including analyses of impurities. In addition, results of several genotoxicity assays using the characterized materials were provided, along with absorption, distribution, metabolism, and excretion data. Data from a two-generation reproductive toxicity study were also provided.

The CIR Expert Panel considered that because PEGs Soy Sterol may be broken down to PEGs and Soy Sterols, the safety test data on the PEGs and Soy Sterols are directly relevant to the safety assessment of PEGs Soy Sterol.

Polyethylene Glycol of various chain lengths (PEGs) has been reviewed previously by the CIR Expert Panel and the Final Report has been published. The following conclusion was reached:

> 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).

Data on plant phytosterols, to the extent that they are representative of soy phytosterols, are also relevant to this safety assessment and have been included in this amended safety assessment.

It was considered unlikely that there would be any toxicity of PEGs Soy Sterol esters that was not present in the two constituents.

CHEMISTRY

Definition and Structure

PEG-*n* Soy Sterol is a polyethylene glycol derivative of sterols found in soybean oil where *n* is the average number of moles of ethylene oxide used in synthesis (Wenninger et al. 2000). These phytosterols (generic term) are structurally similar to cholesterol and mainly consist of sitosterol ($C_{29}H_{50}O$: molecular weight [mw] 414.69), campesterol ($C_{28}H_{48}O$: mw 400.66), and stigmasterol ($C_{29}H_{48}O$: mw 412.67) (Applewhite 1985; Budavari 1989; Tyle and Frank 1991).

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). The general structure of cholesterol, campesterol, sitosterol (β and γ), and stigmasterol are depicted in Figure 1.

PEG-*n* Soy Sterol is also known as PEG-*n* Soya Sterol (Pepe, Wenninger, and McEwen 2002) 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- and -2000 Soya Sterol, respectively (Pepe, Wenninger, and McEwen 2002).

Chemical and Physical Properties

Physical and chemical properties of PEG Soy Sterol are summarized in Table 1.

PEG-5 and -10 Soy Sterol 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 Sterol 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).

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°C 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 reported melting ranges of 73°C to 80°C and 55°C to 58°C, a pH of 4.5 to 7.0 (in 1% aq. dispersion at standard temperature), and a hydroxyl value of 60 to 90 (Lundmark, Chun, and Melby 1976; Nikitakis and McEwen 1990).

PEG-16 Soy Sterol melts at 46°C to 50°C and has an HLB of 15 (Lundmark et al. 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°C 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).

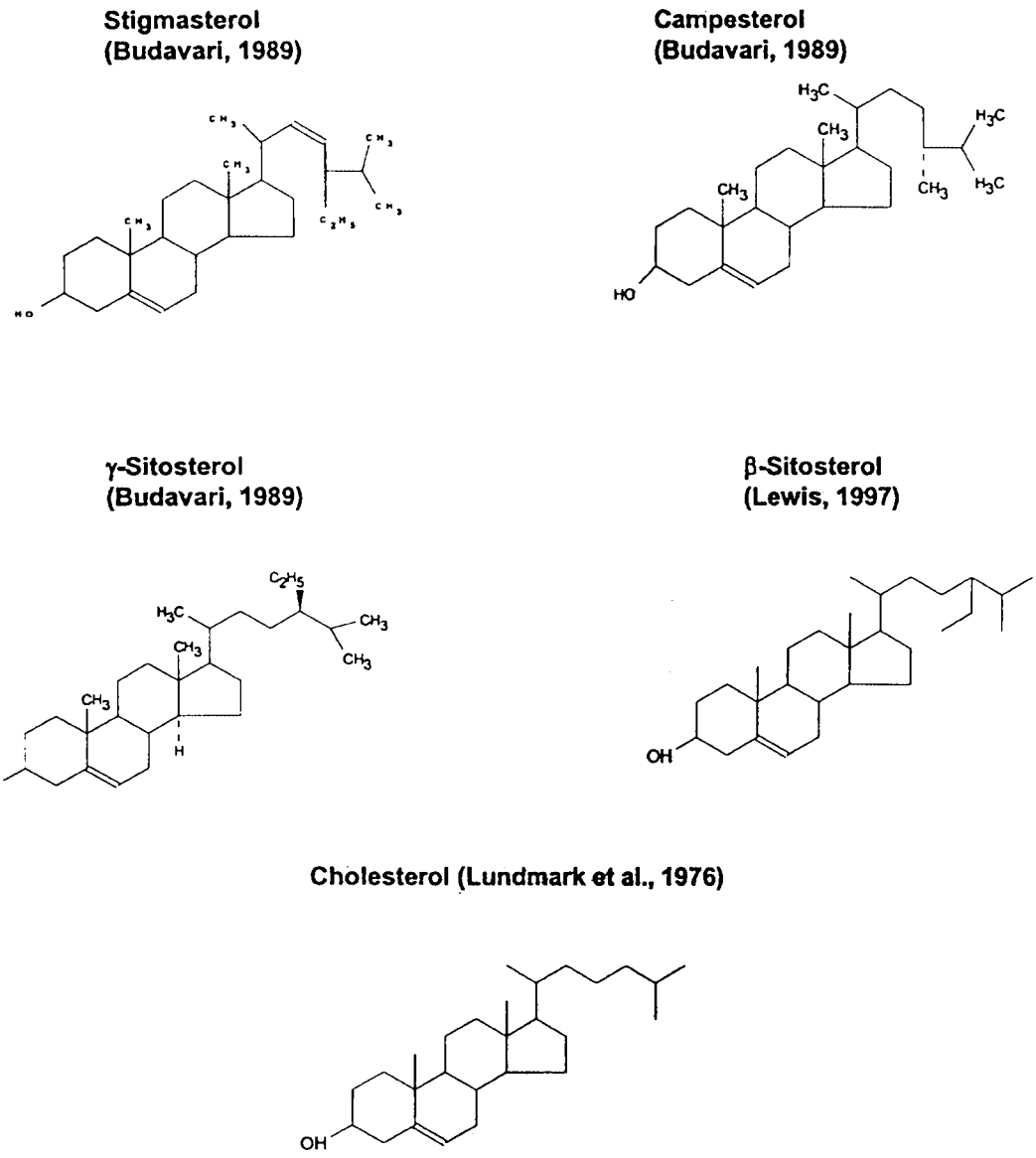

FIGURE 1
The general structure of cholesterol, campesterol, sitosterol (β and γ), and stigmasterol.

Method of Manufacture

Phytosterols

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.

PEGs Soy Sterol

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

TABLE 1
Physical and chemical characteristics of PEGs soy sterol

Characteristics	PEG-5 Soy Sterol	PEG-10 Soy Sterol	PEG-16 Soy Sterol	PEG-25 Soy Sterol	Reference
Physical properties	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 et al. 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 et al. 1976; Nikitakis and McEwen 1990
Melting point	74–88°C	73–80°C (55–58°C)	46–50°C	44–48°C	Lundmark et al. 1976; Nikitakis and McEwen 1990; Tyle and Frank 1991
Hydrophile-lipophile balance	5	12	15	17	Lundmark et al. 1976
pH (1% aqueous dispersion at 25°C)	5.0–7.0	4.5–7.0	—	—	Lundmark et al. 1976; Nikitakis and McEwen 1990
Hydroxyl value	80–110	60–90	70	55	Lundmark et al. 1976; Nikitakis and McEwen 1990
Critical micelle concentration	—	—	0.22%	0.46%	Lundmark et al. 1976

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 microscopy, the compound absorbs at 270 nm and 293 nm in a ratio of ~1.73.

Impurities

PEGs

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). 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 may also contain 1,4-dioxane, a byproduct of ethoxylation (Robinson and Ciurczak 1980). 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).

Soy Phytosterols

In a review of the use of soy sterols, Lundmark, Chun, and Melby (1976) reported that refined soy sterols contain approximately 88% total sterol. Of that percentage, 56% is 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 steroid-like substances (Lundmark, Chun, and Melby 1976).

Swern (1979) summarized the results of analyses of the total (phytosterol esters + free phytosterols), free, and the ester/free ratio in soybean preparations. In 100 mg of alkali-refined soybean oil, there was 0.446 mg total sterol and 0.287 mg free sterol. By subtraction, the phytosterol esters would be 0.159 mg and the ratio of esterified to free sterol was 0.55. In another alkali-refined soybean oil, there was 0.481 mg total sterol and 0.333 mg free sterol. By subtraction, the phytosterol esters would be 0.148 mg and the ratio of esterified to free sterol was 0.44.

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

Phytosterols

Unilever (1998a 1998b) reported on the chemical characterization and stability of a single sample of plant sterols and from samples from five different batches of a single production process. The plant from which these phytosterols were derived was not stated. High-performance liquid chromatography was used to analyze fatty acid composition, gas chromatography/mass spectrometry for sterols, and Fourier transform infrared analysis as a check that the chemical bonds seen were consistent with the chemical species identified. The results presented in Table 2 show that the distribution of specific phytosterols, etc. are remarkably consistent. Reanalysis indicated the samples to be stable for a period of at least 10 months under refrigerated conditions. The fatty acid content of all samples was around 38% and the phytosterols was 62%.

TABLE 2
Chemical characterization of plant sterol material (Unilever 1998a 1998b)

	Distribution of phytosterols (%)	
Phytosterols	Single sample	Five samples from five batches
Brassicasterol	1.1	2.7–3.1
Campesterol	25.8	26.5–27.0
Stigmasterol	21.6	17.4–18.1
β-Sitosterol	48.7	50.8–51.2
β-Sitostanal	1.8	Not given
Cholesterol	0.4	0.2–0.3
Other sterols	0.8	1.2–1.7

In an analysis of another source of phytosterols using the same techniques described above, Unilever (1996a) reported that the principal phytosterols were present as follows: β-sitosterol, 47.9%; campesterol, 28.8%; and stigmasterol, 23.3%. No impurities were found. In an analysis of phytosterol esters, Unilever (1996b) reported that the principal phytosterols were present as fatty acid esters: β-sitosterol, 47.3%; campesterol, 28.1%; and stigmasterol, 24.5%. The distribution of the fatty acid chain lengths was consistent with fatty acids derived from sunflower oil.

Comparison of Phytosterol Compositions from Different Plants

The argument has been made that the available data on phytosterols is relevant to evaluating the safety of the soy phytosterol component of PEGs Soy Sterol (Brock 2000) because of similarity in structure of the phytosterols that have been studied and soy phytosterols. The relevance of phytosterol characterizations as seen above and the safety test data that will follow to soy sterols is a function of the similarity between phyto-sterols, regardless of source. In one case above, for example, the implication is that the source of the phytosterol esters undergoing analysis was sunflower seed oil and a question should be asked about the relevance of those data to soy sterols. Swern (1979) has compiled data from the literature on the percent distribution of phytosterols from 16 common oils. These data are shown in Table 3.

Clearly, campesterol and stigmasterol are present in significant proportions in most of these oils and β-sitosterol is present at a high percentage in all of them. The chemical characterization data in the preceding section shows a distribution of campesterol, stigmasterol, and β-sitosterol that is not substantially different from that shown in Table 3 for soy.

In the safety test data that follows, sections will be included that describe results of studies on phytosterols without reference to the plant source. The implication is that these data are likely relevant to the assessment of the campesterol, stigmasterol, and β-sitosterol components that principally comprise soy phytosterols based on the comparisons in Table 3.

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 (Pepe, Wenninger, and McEwen 2002). These compounds also are used as appearance and consistency modifiers, emollients, viscosity control agents, and pigment dispersion agents (Lundmark, Chun, and Melby 1977).

Table 4 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

TABLE 3
Percent distribution of phytosterols from 16 common vegetable oils (Swern 1979)

	Phytosterol composition					
Oil source	Brassicasterol	Campesterol	Stigmasterol	β-Sitosterol	Δ^7 Stigmastenol	Unknown
Cocoa butter		8–11	24–31	59–62		
Coconut	2	6–9	18–19	69–75		
Corn		10–20	Trace-6	74–89		1
Cottonseed	Trace–1	8		89–91		
Linseed	2	28	10	53	4	
Olive		1–3	2	80–97		18
Palm		20–21	12–13	62–67		
Peanut	1	10–19	6–12	70–76		
Rapeseed	5–19	22–37		52–62		
Rice bran		14–33	3–6	55–63		
Safflower		8–13	4–9	52–57		23
Soybean		15–21	10–24	57–72		1
Sunflower		11–12	8–12	62–75	20	

1 cosmetic formulations, respectively. PEG-30 Soya Sterol was not reported to be used (FDA 1996).

Concentration of use data are no longer required to be submitted to the FDA by the cosmetics industry, but historical data 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). Current concentration of use information was provided by the industry through the Cosmetic, Toiletry, and Fragrance Association (CTFA). 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

TABLE 4
Product formulation data for PEGs Soy Sterol (FDA 1996)

		Total no. of formulations containing ingredient				
Product category	Total no. of formulations in category	PEG-5 Soy Sterol	PEG-10 Soy Sterol	PEG-16 Soy Sterol	PEG-25 Soy Sterol	PEG-40 Soy Sterol
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

TABLE 5
Current concentration of use of PEGs Soy Sterol (CTFA 2000)

Product category	Concentration			
	PEG-5 Soy Sterol (%)	PEG-10 Soy Sterol (%)	PEG-16 Soy Sterol (%)	PEG-25 Soy Sterol (%)
Eye lotion	2		0.2	
Mascara	2	2		
Makeup bases		1		
Foundations		0.8		2
Other eye makeup preparations		0.1		
Hair conditioners (noncoloring)		2		
Shampoos (noncoloring)			0.5	0.5
Tonics, dressings, and other hair-grooming aids		0.4	0.5	
Other makeup preparations		0.05		
Shaving cream		2		
Other shaving preparation products		2		
Face and neck creams, lotions, powders, and sprays (excluding shaving preparations)				0.5
Body and hand creams, lotions, powders, and sprays (excluding shaving preparations)	0.2–2	2		
Moisturizing creams, lotions, powders, and sprays (excluding shaving preparations)		2	0.2	
Paste masks (mud packs)				0.5
Skin fresheners		2		

(CTFA 1997). CTFA (2000) updated those data with the information contained in Table 5.

Noncosmetic

In 1982, polyoxyethylene adducts of mixed phytosterols (with 5 to 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 (Rothschild 1990; EPA 2002).

GENERAL BIOLOGY

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).

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

PEGs

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 digestive enzymes 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. (1990b) 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 1990a). Conversion of campesterol into bile acids was reported in rats (Boberg et al. 1990b). 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 (Ling and Jones 1995). The excretion rate of sitosterol from bile was ten times greater than that of cholesterol (Gould 1955; American Cyanamid Co. 1957).

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 (strain not given) and baboons (Sabine 1977).

Unilever (1997a) traced the fate of radioactively labeled β-sitosterol and β-sitosterol linoleate administered by gavage to male rats. Twenty young, adult male Charles River CD rats (146 to 172 g) were individually housed. Ten animals received the β-sitosterol and 10 received the ester. The vehicle was sunflower oil and delivery was by gavage. Urine and feces were collected and individual CO_2 absorbers filtered the air which was drawn through at a rate of 1 L/min. At 4, 8, 24, 72, and 96 h, one rat from each group was weighed and euthanized for whole body autoradiography. At 96 h, heart blood was collected from the remaining animals and they were euthanized for analysis of radioactivity in internal organs.

The absorption, distribution, metabolism, and excretion of β-sitosterol and β-sitosterol linoleate were similar. Over 90% of the dose was excreted in the feces; less than 0.1% was excreted in the urine. Absorption from the gut was low. What was absorbed ended up in the intestinal lining, liver, lung, and adrenal gland. The highest concentration as well as persistence of the radioactive label was in the adrenal gland. In the gut, the β-sitosterol linoleate was hydrolyzed to release free sterol, but esterification with fatty acids present in the gut with free sterol also occurred.

In another study of the fate of radioactively labeled β-sitosterol and β-sitosterol linoleate, Unilever (1997b) evaluated the impact of a sunflower oil vehicle versus a coconut oil vehicle. In the sunflower oil part of the study, six young male and six young female Charles River CD rats (152 to 174 g) were dosed by gavage. At 24, 48, and 96 h, one animal of each sex was weighed and euthanized for whole body autoradiography. Urine and feces were collected. In the coconut oil portion of the

study, five young male and five young female Charles River CD rats (163 to 193 g) were also individually housed. At 24 h, the rats were weighed and euthanized for analysis of radioactivity in internal organs.

As in the previous study, β-sitosterol and β-sitosterol linoleate were poorly absorbed. The radioactive label was slightly higher in females. The adrenal gland showed the greatest amount and retention of radioactive label, but the ovaries, bone marrow, liver, intestinal lining, and spleen were also target organs for what radioactive label was absorbed. The testes in male rats was not a target organ. There was extensive hydrolysis of the ester and there was esterification of which varied as a function of the vehicle; there was more esterification with the sunflower oil than with the coconut oil. β-Sitosterol fatty acid esters with fatty acid components found only in coconut oil and not in the diet confirmed that esterification of free β-sitosterol.

Many of the results from the above Unilever (1996a, 1996b, 1997a, 1997b) studies were combined with other data on the influence on absorption of specific phytosterols (cholesterol, β-sitosterol, β-sitostanol, campesterol, or stigmasterol) on absorption, distribution, metabolism, and excretion in the rat in a report by Sanders et al. (2000). Using similar methods, the essential findings from above were repeated: the total absorption of phytosterols is low (cholesterol by contrast was well absorbed); female animals absorb more than males; and the largest amout of radioactivity from labeled phytosterols ends up in the adrenal gland, although the ovaries and intestinal epithelia also have significant levels.

Effects 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, 1993; Tvrzická et al. 1991) 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 (Heinemann et al. 1991).

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 5α 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. The authors suggested that hydrophobic plant sterols with a high affinity and low capacity for micellar binding could have effectively displaced cholesterol from micellar binding (Heinemann, Axtmann, and von Bergmann 1993).

In a study by Sato et al. (1995), cultured in the presence of plasma lipoproteins, cells acquired cholesterol through receptor-mediated endocytosis of low-density lipoprotein (LDL). LDL-derived cholesterol esters in lysosome 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 Effects of Phytosterols

Exposure to 0.7 mmol/L (incorporated into liposomes) sitosterol for 72 h caused contraction of human umbilical vein

endothelial cells in vitro and an increased release of intracellular lactate dehydrogenase. At 96 h, partial detachment from the substrate was observed. In addition, 0.35 mmol/L of sitosterol caused pertubation of the endothelial cells at 96 h (Boberg, Pettersen, and Prydz 1991).

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). A β-sitosterol isolate had antiatherogenic effects through inhibition of platelet aggregation (Pollak 1985). β-Sitosterol isolated from piper betle leaves (concentration not given) inhibited human platelet aggregation induced by arachidonic acid, platelet-activating factor, and adenosine diphosphate (ADP) (Saeed et al. 1993). Sitosterol can have potent anti-inflammatory, antibacterial, and antifungal activities (Padmaja, Thankamany, and Hisham 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).

ANIMAL TOXICOLOGY

Acute Toxicity

PEGs Soy Sterol

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).

North American Science Associates, Inc. (1987a) evaluated the dermal toxicity of a liquid eyeliner containing 2% PEG-5 Soy Sterol using ten New Zealand white rabbits. Each rabbit weighed between 2.5 to 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 h, and the collars at test termination. The rabbits were observed for signs of toxicity immediately after treatment, at 4 h, 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 euthanized 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 (North American Science Associates, Inc. 1987a).

PEGs

Toxicity studies using rats, rabbits, and dogs indicate that PEGs have low oral and dermal toxicity. In general, the larger molecular weight PEGs appear to be less toxic than the smaller molecular weight PEGs in oral studies. Acute oral LD_{50} 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 20 ml/kg of undiluted PEG-6 or 40% PEG-20M (Andersen 1993).

Short-Term Toxicity

PEGs

Andersen (1993) reported that there was no evidence of toxicity, with the exception of transient, mild erythema, in rabbits that received daily topical applications of PEG-75 or PEG-20M (0.8 g/kg/day) for 30 days.

Evidence of systemic toxicity was found, however, in a study designed as an animal model to study the effects of PEG-based antimicrobial creams in burn patients. Elevated total serum calcium, elevated osmolality gap, high anion gap metabolic acidosis, and renal failure resulted in rabbits that received repeated dermal applications of an antimicrobial cream containing 63% PEG-6, 5% PEG-20, and 32% PEG-75 to two paravertebral skin excisions (2.5 $\times$ 15 cm), followed by wound dressings (changed every 12 h) for 7 days (Andersen 1993).

Phytosterols

Laraki et al. (1993) studied the effects of phytosterols in the diet of rats. Male adult Wistar rats weighing 215 $\pm$ 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 (see Table 6).

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 $\pm$ 0.1 g/day. The rats were euthanized and the livers sampled post mortem, washed, and frozen. Enzymatic activities of acetyl–coenzyme A (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 6 presents enzyme activities determined in 12 rats in each treatment group. Acetyl-CoA carboxylase activity was significantly increased in group 4 (12 mg/day cholesterol and 48 mg/day phytosterol) compared to group 1 (12 mg/day cholesterol only); and decreased in groups 6, 7, and 8 (24 mg/day

TABLE 6
Diet composition in a rat short-term feeding study (Laraki et al. 1993)

	Group							
	1	2	3	4	5	6	7	8
Diet								
Cholesterol (mg/day)	12	12	12	12	24	24	24	24
Phytosterol (mg/day)	—	12	24	48	—	24	48	96
Phytosterol: Cholesterol Ratio	0	1	2	4	0	1	2	4
Liver enzyme activities (means ± SEM)[a]								
Acetyl-CoA carboxylase	538 ± 109	536 ± 43	522 ± 154	664 ± 123*	741 ± 199	235 ± 59**	225 ± 92***	233 ± 99***
Malic enzyme	0.39 ± 0.16	0.39 ± 0.07	0.30 ± 0.06	0.32 ± 0.06	0.44 ± 0.13	0.16 ± 0.04*	0.16 ± 0.04*	0.16 ± 0.03*
Glucose-6-phosphate dehydrogenase	1.33 ± 0.20	1.32 ± 0.20	1.26 ± 0.15	1.48 ± 0.26	2.39 ± 1.23	0.46 ± 0.19	0.57 ± 0.19	0.62 ± 0.15
Liver fatty acids and sterols (means [wet weight] ± SEM)[a]								
Fatty acids (g/100 g)	3.1 ± 0.3	3.5 ± 0.3	3.6 ± 0.3	3.0 ± 0.2	7.8 ± 2.8	2.8 ± 0.3***	2.7 ± 0.3***	3.0 ± 0.3***
Cholesterol (mg/100 g)	216.4 ± 1.5	218.7 ± 3.1	210 ± 2.8*	199.9 ± 1.9***	301.0 ± 13.3	239.4 ± 9.0***	209.4 ± 6.1***	203.4 ± 7.7***
Campesterol (mg/100 g)	0.81 ± 0.04	1.86 ± 0.17***	1.96 ± 0.15***	2.19 ± 0.16***	0.87 ± 0.07	4.68 ± 0.51***	5.03 ± 0.50***	8.00 ± 0.66
β-Sitosterol (mg/100 g)	2.95 ± 0.20	3.96 ± 0.23*	3.74 ± 0.16***	3.88 ± 0.24***	2.92 ± 0.06	8.84 ± 0.25***	10.90 ± 0.27***	12.86 ± 0.75***

[a]Student's t test significance ($^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$) versus the group receiving the same cholesterol supplementation without cholesterol.

cholesterol with 24, 48, or 96 mg/day phytosterol, respectively), compared to group 5 (24 mg/day cholesterol only). Malic enzyme activity was decreased in groups 6, 7, and 8 compared to group 5. Glucose-6-phosphate dehydrogenase activity was not decreased significantly in any phytosterol treatment groups.

Analysis of liver fatty acid and sterol contents (see Table 6) demonstrated increases in fatty acids in groups 6, 7, and 8 compared to group 5 (see Table 6). Cholesterol decreases in the liver were seen in groups 3 and 4 compared to group 1; and in groups 6, 7, and 8 compared to group 5. Campesterol increases were seen in groups 2, 3, and 4 compared to group 1; and in groups 6 and 7 (but **not** in group 8, although the value reported in Table 6 would appear to be significant) compared to group 5. β-Sitosterol increases were seen in groups 2, 3, and 4 compared to group 1; and in groups 6, 7, and 8 compared to group 5.

The authors noted (see Table 6) that liver fatty acids increased by a factor of 2.5 in group 5 (24 mg/day cholesterol) compared to group 1 (12 mg/day cholesterol) and that addition of phytosterol to the high cholesterol diet (groups 6, 7, and 8) reduced the liver fatty acid levels to that seen in group 1 (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, aspartate aminotransferase, and alanine transaminase) 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

PEGs

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).

Phytosterol Esters

Hepburn, Horner, and Smith (1999) presented the results of a 90-day oral toxicity study of phytosterol esters. Groups of 20 male and 20 female Wistar derived rats were fed diets containing phytosterol esters (62% phytosterols and 38% fatty acids). Composition data were presented earlier in Unilever (1998a) and the mix of phytosterols shown in the first column of Table 2. Fatty acids were linoleic acid (64.6%), oleic acid (21.6%), stearic acid (4.1%), and palmitic acid (9.6%). Five dose groups received 0%, 0.16%, 1.6%, 3.2%, and 8.1% for 90 consecutive days. Clinical observations, body weights, and food and water consumption were determined during the exposure. At the end of the exposure period, the animals were euthanized; cardiac blood samples were taken, organs (adrenal gland, brain, epididymides, heart, kidney, liver, spleen, testes, thymus) were weighed, and a standard comprehensive set of tissues taken for histological examination.

Diets containing plant phytosterol esters at all levels were well tolerated. During the exposure, there were no changes in body weight gain, food and water consumption, or clinical signs. There were slightly reduced platelet counts and a small decrease in prothrombin time in female rats at all dose levels compared to controls. There was a small increase in the activated partial thromboplastin time in male rats at the 3.2% and 8.1% dietary levels. Other small increases in plasma albumin, phosphorus or magnesium levels, and certain serum enzymes were reported for males and/or females at the 1.6%, 3.2%, and 8.1% dietary levels. No treatment-related effect was seen with organ weights and histological examination revealed no evidence of systemic toxicity. Absent any organ effects, the small hematology and blood chemistry variations were not considered of toxicological significance (Hepburn, Horner, and Smith 1999).

Chronic Toxicity

PEGs

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 70–212 days (General Mills, Inc. 1979).

Dermal Irritation and Sensitization

PEGs Soy Sterol

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 h later, and the treated sites were scored at that time and at 72 h. The primary irritation index (PII) score was

0.50 for PEG-10 Soy Sterol and 0 for PEG-5, -16, and -25 Soy Sterol (Warf Institute, Inc. 1974; Henkel Corp. 1995).

PEG-5 Soy Sterol at 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 × 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 h, and the skin sites were rinsed with tap water. The sites were evaluated at 24 and 72 h 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.

PEGs

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 h 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

PEGs Soy Sterol

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 h 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).

PEGs

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).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

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.

Estrogenic Effects of Phytosterols and Phytosterol Esters

Malini and Vanithakumari (1992) reported that β-sitosterol acted as an effective estrogen-like 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, Malini and Vanithakumari (1993) administered β-sitosterol 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 to 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/100 g/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 sevenfold 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).

In a study on the content and estrogen receptor of phytoestrogens in various foods, herbs, and spices, Zava, Dollbaum, and Blen (1998) reported 8 μg of estradiol equivalents per 200 cm^3 of soy milk extract in an estrogen receptor binding assay. Activity was extracted from soy milk with ethanol/water (50:50) for 2 days. These authors also report the results of ingestion of 200 cm^3 of soy milk on the estrogen receptor binding activity of saliva of four volunteers. Saliva was extracted with diethylether, dried to a precipitate, and reconstituted in growth medium for the binding assay. At least one individual had residual estrogen receptor binding 9 to 10 h after ingestion of the soy milk (all had estrogen receptor binding in saliva 1 to 4 h after ingestion).

Baker et al. (1999), however, found no evidence of estrogenic activity of phytosterols in a competitive estrogen-binding assay, a recombinant yeast assay, and in a juvenile rat uterotrophic assay. The phytosterols (see Table 2) used were described earlier (Unilever 1996a).

In the estrogen-binding assay, estrogen receptors isolated from 10-week-old female Wistar rats were prepared and incubated with estradiol (positive control) or test substances. Estradiol produced a concentration dependent (from 10^{-13} M to 10^{-6} M) binding, whereas phytosterols had no binding at concentrations of 10^{-7} M to 10^{-4} M.

In the recombinant yeast assay, the DNA of the human estrogen receptor and plasmids with the gene for the β-galactocidase enzyme controlled by estrogen responsive sequences are incorporated into yeast cells. In the presence of estrogens, the enzyme is produced by the yeast cells and its activity measured. Coumestrol, a known weak phytoestrogen, was included as a test substance to calibrate the sensitivity of the assay. Estradiol was used as a positive control and the activity of a phytosterol mix (see Table 2) and β-sitosterol alone. No enzyme activity was induced by the phytosterol mix ($\sim 10^{-10}$ M to 10^{-4} M) or β-sitosterol ($\sim 10^{-7}$ M to 10^{-4} M) alone. Both estradiol (maximum induction at $\sim 10^{-9}$ M) and coumestrol (maximum induction at $\sim 10^{-7}$ M) were active.

The juvenile rat uterotrophic assay utilized 21- to 22-day-old Wistar rats. Test materials were dissolved or suspended in arachis oil. Groups of 10 animals received a single dose of 0, 5, 50, or 500 mg/kg in a dosing volume of 10 ml/kg on each of 3 successive days by gavage. Estradiol was the positive control. Daily clinical observations were made over the period of dosing and body weights recorded at the same time. Animals were euthanized 24 h after the last dosing and the absolute uterine weight determined as the utertrophic endpoint. Arachis oil (vehicle), phytosterols, phytosterol esters, cholesterol, and cholesterol palmitate failed to produce any increase in uterine weight. The positive control produced a significant increase when compared to these test materials. Coumestrol was less effective in producing increased uterine weight compared to estradiol, but it did produce a clear dose-related increase.

The authors concluded that the well-defined phytosterol materials used in these studies were not estrogenic. They suggested that estrogenic activity seen in other studies may relate to actual phytoestrogens present in crude extracts (Baker et al. 1999).

Reproduction Studies

PEGs

Andersen (1993) described an oral toxicity study in which PEG-75 was given to rats (in drinking water) for 90 days. Doses above 0.23 g/kg/day were associated with testicular tubule degeneration, and scant or degenerated sperm, but historical controls using this strain (strain not given) reportedly had similar effects.

In a chronic oral study, PEG-75 was given to Wistar albino rats in drinking water at doses up to 0.062 g/kg/day and PEG-6–32 was given to a separate group at doses up to 1.69 g/kg/day. Animals were allowed to breed during the study and records were kept of the F_1 and F_2 generations. No changes or adverse responses to either compound occurred in the three generations (Andersen 1993).

Phytosterols

In a study by Malini and Vanithakumari (1991), male Wistar rats were injected subcutaneously with 0.5 to 5 mg/kg/day β-sitosterol for 16, 32, or 48 days. They found 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 laparotomised 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 $\pm$ 42, and the sperm concentration of rats given the phytosterol was 366 $\pm$ 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).

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).

Phytosterol Esters

Noting the estrogenic activity of phytosterols reported by Malini and Vanithakumari (1993) above, as well as by Mellanen et al. (1996) and Rosenblum et al. (1993), Waalkens-Berendsen et al. (1999) conducted a two-generation reproduction study in rats receiving phytosterol esters in their diet. Four groups of Wistar outbred rats (28 animals of each sex in each group) were fed diets with 0%, 1.6%, 3.2%, or 8.1% phytosterol esters. The composition of the phytosterol esters was 62% phytosterol and 38% fatty acid. The distribution of phytosterols is as shown in the first column of Table 2.

Fatty acids were linoleic acid (64.6%), oleic acid (21.6%), stearic acid (4.1%), and palmitic acid (9.6%). At the end of a 10-week premating period, one male and one female (from the same exposure group) were housed together and the female checked each morning for evidence of mating. Mated F_0 females were caged separately. The morning after birth was considered postnatal day 1. On postnatal day 21, F_1 pups were weaned and 28 males and 28 females selected at random. These animals ($\sim$5 weeks old) began a premating period and the mating was again performed as above to produce the F_2 generation. Throughout the study, animals were observed daily for clinical signs and abnormal behavior. Body weights were determined weekly. Food consumption was measured weekly.

Reproductive performance included the calculated indicies of mating, fertility, fecundity, gestation, live births, postnatal viability, and lactation, and the sex ratio of pups was determined. Animals not utilized in the ongoing reproductive study were euthanized and subjected to thorough necropsy, including examination of the reproductive organs of the F_0 and F_1 males that failed to sire. The reproductive organs of the nonmated and nonpregnant females of the low- and mid-dose groups were also examined. At study termination, all surviving parental animals in the F_0 and F_1 generations were also euthanized and subjected to thorough necropsy.

There were no treatment-related clinical observations. Body weights of male rats of the F_0 and F_1 generations in high-exposure groups were decreased (compared to control males) in weeks 2 to 7 but not consistently at other times. Weight decreases in the low-exposure group occurred at different times. No body weight differences were seen in females. Food consumption was likewise not consistently different between control and exposed groups. There were no treatment-related organ weight changes, nor were there any macroscopic or histological evidence of systemic toxicity in those organs, including reproductive organs. There was no effect of phytosterol esters on fertility, reproductive performance, sexual maturation, oestrous cycle length, or precoital time. Pup mortality on a per litter basis was not affected by exposure to phytosterol esters in the diet. There were no differences in pre/postimplantation loss, stillborn pups, and litter size and weight. Overall, the authors concluded that dietary phytosterol esters up to 8.1% had no effect on any parameter of reproduction or fertility (Waalkens-Berendsen et al. 1999).

GENOTOXICITY

PEGs Soy Sterol

PEG-5 Soy Sterol at 8-5000 μg/plate (in Tween 80/bidist 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).

PEGs

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).

Phytosterols and Phytosterol Esters

Huntington Life Sciences Ltd. (1996a, 1996b) evaluated the mutagenicity of phytosterols in *S. typhimurium* strains TA1535, TA1537, TA98, and TA100 with and without metabolic activation. The phytosterol preparation was described earlier (Unilever 1996a). No toxicity at concentrations up to and including 5000 μg/plate, so that level was used in the mutagenicity assay. S9 liver preparations from Aroclor 1254–induced rats was used for metabolic activation. In the absence of S9 activation, *N*-Ethyl-*N*′-nitro-*N*-nitrosoguanidine was used as a positive control for TA1535 and TA100, 9-aminoacridine for TA1537, and 2-nitrofluorene for TA98. With S9 activation, 2-aminoanthracene was used as the positive control. Tetrahydrofuran was used to dissolve the test substances and served as the negative control. No significant increases in mutations with or without metabolic activation was seen with the phytosterol preparation used.

Likewise, no significant increases in mutations with or without metabolic activation was seen with the phytosterol ester preparation (as described by Unilever 1996b).

This same laboratory evaluated the mutagenicity of plant sterols using the same assay described above (Huntington Life Sciences Ltd. 1998). The plant phytosterol preparation was described earlier (Unilever 1998a). No significant increases in mutations with or without metabolic activation was seen with the plant sterols preparation used.

A metaphase chromosome analysis of human lymphocytes in vitro exposed to phytosterols and phytosterol esters was conducted by Huntington Life Sciences Ltd. (1997a, 1997b). The phytosterols and phytosterol esters were characterized earlier (Unilever 1996a, 1996b). Two tests were done with each test material. In the first test, a range of concentrations of test materials were added to one set of duplicate cultures. Acetone was used as a solvent and control. Mitomycin C was added. The second set of cultures received S9 liver fraction and the same concentrations of test materials and control as in the first set (adjusted to accommodate the extra volume of the S9 mix).

Exposure was 3 h for this second set, at which point the cells were washed and resuspended in fresh medium and incubated for 18 h. The first set of cultures was incubated for 21 h with the test material. Cells were harvested, Colcemid was added to arrest mitotic activity, the cells further incubated for 2 h and then washed and treated prior to placement on microscope slides for visual examination. Metaphase figures were examined (~100 from each culture) at 1000× magnification. In the second test, the procedure was similar, except that some cultures were harvested at 21 h and others allowed to continue for 45 h. The concentrations of phytosterols and phytosterol esters tested in each regimen with notes regarding formation of precipitate are shown in Table 7.

TABLE 7
Concentrations of phytosterols and phytosterol esters used in chromosome analysis (Huntington Life Sciences Ltd. 1997a, 1997b)

	Concentration (μl/ml)					
	Without metabolic activation			With metabolic activation		
	First test	Second test		First test	Second test	
Test agent	21 h	21 h	45 h	21 h	21 h	45 h
Untreated	—	—	—	—	—	—
Acetone	10	10	10	10	10	10
Phytosterols	1.3			1.3		
	2.5			2.5		
	5			5		
	10			10		
	20			20		
	40[a]	40[b]	40[b]	40	40[b]	40[b]
	80[b]	80[a]	80[a]	80[a]	80[b]	80[b]
	160[b]	160[a]	160[a]	160[a]	160[a]	160[a]
Phytosterol	.78			.78		
esters	1.56			1.56		
	3.13			3.13		
	6.25			6.25		
	12.5			12.5		
	25	25		25	25	
	50[a]	50[a]	50[a]	50	50	
	100[a]	100[b]	100[b]	100[a]	100[a]	100[a]

[a]Precipitate on dosing, still apparent at end of treatment.
[b]Precipitate on dosing, not apparent at end of treatment.

No significant incidence of chromosome aberrations or change in mitotic index was seen in any of the tests with either phytosterols or phytosterol esters in this test at any concentration, with or without metabolic activation (Huntington Life Sciences Ltd. 1997a, 1997b).

Covance Laboratories Ltd. (1999a) determined unscheduled DNA synthesis in the liver of Han Wistar rats dosed orally with plant sterol esters. In each of two studies, groups of five male rats (196 to 231 g) received corn oil (vehicle control), 800 or 2000 mg/kg plant sterol esters, or positive control. In one study 2-acetamidofluorene was the positive control and dimethylnitrosamine in the other. The composition of the plant sterol ester was described earlier (Unilever 1998b). In all cases, the dose volume was 10 ml/kg delivered by oral gavage.

In one study, animals were euthanized 10 to 14 h post dosing and in the other at 2 to 4 h. The liver of each rat was prepared by washing it free of blood and treating with collagenase. The liver was harvested and hepatocytes separated out. Hepatocyte suspensions were placed into wells containing a glass coverslip, incubated at 37°C for at least 90 min to allow adhesion to the coverslip. Adhered cells were washed with serum free medium and then incubated with the same medium with ^{3}H-thymidine added for 4 h. The cells were again washed and incubated overnight in the serum free medium. Incorporation of radioactivity in the cells was determined by counting grains in autoradiographic images of the cells. No evidence of unscheduled DNA synthesis in rat liver hepatocytes exposed in vivo to plant sterol esters was found (Covance Laboratories Ltd. 1999a).

This same laboratory (Covance Laboratories Ltd. 1999b) examined the induction of bone marrow micronuclei in outbred Crl:HanWist male rats given plant sterol esters by oral gavage. Groups of eight rats were treated with corn oil (vehicle control), 500, 1000, or 2000 mg/kg of plant sterol esters, or cyclophosphamide (positive control). The composition of the plant sterol ester was described earlier (Unilever 1998b). In all cases, the dose volume was 10 ml/kg, given once daily on each of 2 consecutive days. Animals were euthanized 24 h after the second administration. A single femur of each animal was removed, cleaned, prepared, and the bone marrow flushed out with fetal bovine serum (2 ml). Bone marrow cells were concentrated by centrifugation and smeared on microscope slides. The slides were allowed to air dry, then fixed and rinsed. A second fixation and rinsing was done before final drying.

Polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs) were determined in at least 1000 cells. Continued counting of PCEs was done until a total of 2000 PCEs were counted. All PCEs containing micronuclei were tabulated. Cells from rats dosed with plant sterol esters had the same PCE/NCE ratio as the vehicle control. The frequencies of micronucleated PCEs (group means) were similar to controls. The authors concluded that plant sterol esters at doses up to 2000 mg/kg did not

induce micronuclei in rat bone marrow cells (Covance Laboratories Ltd. 1999b).

CARCINOGENICITY

PEGs

PEG-8 was used as a solvent control in a number of oral studies intended to evaluate the carcinogenicity of other chemicals. In those studies, no tumors were observed when the solvent was administered orally to mice (30 weeks of dosing), intraperitoneally to rats (6 months of dosing), subcutaneously (20 weeks of dosing—rats; 1 yr of dosing—mice), or when injected into the gastric antrum of guinea pigs over a period of 6 months (Andersen 1993).

Antitumorigenic Effects

Phytosterols

Ling and Jones (1995) reported that evidence is available to suggest that phytosterols inhibit the induction of tumors in animals.

Yasukawa et al. (1991) demonstrated that 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 min 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).

Yasukawa et al. (1991) also hypothesized that 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 min 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 euthanized by cervical dislocation. The epidermis of the treated skin was separated by brief heat treatment, and ODC activity was determined in the soluble epidermal supernant 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 $\pm$ 0.23; sitosterol, 1.3 $\pm$ 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 coadminsitered 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 (Raicht et al. 1980). 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 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 tumors/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 tumors/rat and 1.3 tumors/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 rats (15%) had invasive carcinomas in the sterol plus carcinogen group compared to 5 of 71 rats (7%) in the carcinogen alone group (Raicht et al. 1980).

Cell Proliferation Effects

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 (Janezic and Rao 1992). 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 euthanized after two 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 ($ED_{50} \geq 20$ μg/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 euthanized. 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

PEGs Soy Sterol

Biosearch Incorporated (1992a) patch-tested 12 volunteers, aged 27 to 54 years, with a mascara containing 2% PEG-5 Soy Sterol in a modified Draize repeat-insult patch test (RIPT) to determine irritation potential. On a Friday, 0.75 × 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 h. The procedure was repeated on the following Monday, Tuesday, and Wednesday. The skin sites were evaluated 48 h after the final application. All subjects completed the study.

No evidence of skin irritation was observed.

Biosearch Incorporated (1992b) performed a different modified Draize RIPT using the same formulation and 84 subjects, 75 of whom completed the study. 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 h. 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 h, and the site was examined at 24 h, 48 h, and 72 h. No evidence of irritation or sensitization were observed.

PEGs

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 h 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

PEGs Soy Sterol

Biosearch Incorporated (1987) used a Schwartz-Peck Prophetic Patch Test to determine primary skin irritation of a liquid eyeliner containing 2% PEG-5 Soy Sterol. 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 h. Simultaneously, ~0.1 g of the material was applied to the volar forearm as an open patch. The exposed skin sites were examined 48 h 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 min. After the second insult application, the closed patch site was irradiated. The application sites were reexamined after 48 h 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 to 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 h, the patches were removed and the skin sites were examined. This procedure was repeated three times a week for three and a half 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 h. In addition, the closed patch was irradiated for 1 min using a UV light source (3650 Å) after the first, fourth, seventh, tenth, and

eleventh applications. The subjects were examined 48 h later to determine if photosensitization had occurred. During this study, no signs of primary irritation, sensitization, or photosensitization were observed.

Ten female volunteers (25 to 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 to 400 nm). For exposure to UVA only, a filter was used to eliminate UVB wavelengths (290 to 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 min 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 h, 48 h, and 72 h. 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 was 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 three consecutive weeks, and remained in place for 24 h 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 h, 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 h, 48 h, and 72 h after irradiation. No evidence of photosensitization was observed.

Comedogenicity

PEGs Soy Sterol

The Educational & Research Foundation, Inc. (1991a) used 62 female subjects to evaluate the comedogenicity of SPF 15 liquid foundation containing 0.75% PEG-5 Soy Sterol and 1.5% PEG-25 Soy Sterol. 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 non-acne 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 (Educational & Research Foundation, Inc. 1991a).

In a second (45-day) study, the Educational & Research Foundation, Inc. (1991b) evaluated an SPF 15 liquid foundation (0.75% PEG-5 Soy Sterol, 1.5% PEG-25 Soy Sterol) using 56 female panelists, 52 of whom completed the study.

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 panelists 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 (Educational & Research Foundation, Inc. 1991b).

Ocular Irritation

PEGs Soy Sterol

North Cliff Consultants, Inc. (1992) evaluated the ocular irritancy of a mascara containing 2% PEG-5 Soy Sterol using 60 female subjects. 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, nine 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 of whom were contact wearers (North Cliff Consultants, Inc. 1992).

Biosearch Incorporated (1988) evaluated the cutaneous effects of an eyeliner containing 2% PEG-5 Soy Sterol during a clinical usage study using 60 female subjects between the ages of 18 and 35. 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 lens 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 that 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 (Biosearch Incorporated 1988).

Miscellaneous Effects

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. PEGs Soy Sterols are used in a wide variety of cosmetic products. Recent data supplied by industry indicated that PEG-5 Soy Sterol was used at concentrations up to 2%.

Few data were avaliable on the PEGs Soy Sterol. Because these chemicals may easily be hydrolyzed into the component PEGs and soy sterols, data on the PEGs, soy sterols, and phytosterols in general were included because they were considered relevant 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 campesterol, stigmasterol, and β-sitosterol. The distribution of sterols found in oils derived from common plants is similar, with β-sitosterol comprising the major component. Analysis of production batches of phytosterols demonstrated consistency among batches and stability over time.

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% to 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. β-Sitosterol and the ester, β-Sitosterol linoleate, in particular, have been shown to be poorly absorbed in the gut. A feature of the dynamics in the gut is that the ester may be hydrolyzed and that free sterol may be esterified with linoleate or any other fatty acid present in a dynamic equilibrium.

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.

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 h, and an increased release of intracellular lactate dehydrogenase was observed. At 96 h, 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-5–25 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.

In an animal model designed to study the potential toxicity of repeated applications of a PEG-based antimicrobial cream to burn patients, renal failure was observed 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 7 days. These data were consistent with the clinical picture in which burn patients that received PEG-based topical antimicrobial ointments had renal toxicity and contact dermatitis.

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 ($\leq$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.

In a 90-day oral toxicity study in rats, diets containing plant phytosterol esters at all levels (maximum 8.1%) were well tolerated. Some small hematology and blood chemistry variations from the controls were noted. No treatment related effect was seen with organ weights and histological examination revealed no evidence of systemic toxicity. Absent any organ effects, the small hematology and blood chemistry variations were not considered of toxicological significance.

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-5–25 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. Transient, mild erythema was observed when rabbits were given daily topical applications of 0.8 g/kg/day PEG-75 or PEG-20M for 30 days.

In clinical studies, PEG-5 Soy Sterol at concentrations up to 2% in formulation did not cause dermal or ocular irritation, dermal sensitization, or photosensitization. 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.

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-6-32 and PEG-75.

Although monoalkyl ethers of ethylene glycol are reproductive toxins and teratogenic agents, given the methods of manufacture of the PEGs Soy 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, and as such, are chemically different from alkyl ethers. It is considered unlikely that there would be monoalkyl ethers of ethylene glycol in PEGs Soy Sterol which would cause reproductive or developmental effects based on their structural characteristics. β-Sitosterol was an effective estrogen-like 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. Other studies of well-characterized phytosterols and phytosterol esters demonstrated no effect in an estrogen-binding study, a recombinant yeast assay for estrogen or estrogen-like activity, or a juvenile rat uterotrophic assay for estrogen or estrogen-like activity. Sulfates of β-sitosterol acted 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.

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. A two-generation reproduction study of phytosterol esters in the diet of rats (maximum 8.1%) failed to produce any treatment-related adverse 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. Phytosterols and phytosterol esters were not genotoxic, with or without metabolic activation, in the Ames assay, a human lymphocyte chromosome damage assay, an unscheduled DNA synthesis assay, or a rat bone marrow micronucleus assay.

PEG-8, as a solvent control in studies of suspected carcinogenic agents, 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. Such short duration studies are of utility in assessing the carcinogenicity of only the most potent carcinogens.

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*-methylnitrosourea (by cannulation) and ß-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 μg/ml).

DISCUSSION

The CIR Expert Panel considers that the safety of PEGs Soy Sterol ingredients can be evaluated by reviewing the data available on the ingredients themselves, supplemented by the available data in a previous review of PEGs and the available data on soy phytosterols. In addition, because of the similarities between the composition of soy phytosterols and plant phytosterols in general, the available test data on plant phytosterols is also relevant. Also, no particular toxicity, not seen with the two constituents, would be expected from the PEGs Soy Sterol esters.

Although no dermal absorption data were available, oral studies demonstrate that phytosterols and phytosterol esters are not significantly absorbed and do not result in systemic exposure. Some small amounts did appear in the ovaries, however. One of the Panel's concerns in the original safety assessment of

these ingredients was the potential presence of free phytosterols and β-sitosterol, which could have antiestrogenic, antiprogestational, gonadotrophic, antigonadotrophic, and antiandrogenic effects in PEG sterols. These concerns have been alleviated by the extensive data showing that well-defined phytosterols and phytosterol esters are not estrogenic and do not pose a hazard to reproduction. Likewise, the Panel noted the absence of impurities in plant phytosterols and phytosterol esters and extensive data demonstrating the absence of any genotoxicity in bacterial and mammalian systems, and mitigating against the possibility of any carcinogenic effect with those same well-characterized materials.

The Panel noted that, in clinical tests, PEGs Soy Sterol were not irritating, did not sensitize or photosensitize, produced transient ocular irritation, and were not comedogenic. The CIR Expert Panel, however, was concerned about the sensitization potential of the PEG-5, -10, -16, -25, 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. PEGs were determined to be the causitive agents in both animal and human studies. No evidence of systemic toxicity or sensitization was found in studies with intact skin. Because PEGs Soy Sterol ingredients may be hydrolyzed to release PEGs, these data were of concern for this safety assessment. The cosmetic industry is advised, therefore, to avoid using PEGs Soy Sterol in cosmetic formulations that may 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.

CONCLUSION

On the basis of the available information in this report the CIR Expert Panel concludes that the PEG-5, -10, -16, -25, and -40 Soy Sterol are safe as used in cosmetic products.

REFERENCES

American Cyanamid Co. 1975. Beta-sitosterol, fats, and arteriosclerosis. Submitted by the Food and Drug Administration (FDA) in response to an FOI request dated 3-15-96.

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.

Applewhite, T. H., ed. 1985. *Bailey's industrial oil and fat products*, Vol. 3, 4th ed., 55–58. New York: John Whiley & Sons.

Argus, M. F., J. C. Arcos, and C. Hoch-Ligeti. 1965. Studies on the carcinogenic activity of protein-denaturing agents: hepatocarcinogenicity of dioxane. *J. Natl. Cancer Inst.* 35:949–958.

Baade, S. T., and C. G. Mueller-Goymann. 1994. Lidocaine and soyasterole-PEG-16-ether—investigations on the interaction between an amphiphilic drug and a nonionic surfactant in aqueous solution. *Colloid Polym. Sci.* 272:228–235.

Baker, V. A., P. A. Hepburn, S. J. Kennedy, P. A. Jones, L. J. Lea, J. P. Sumpter, and J. Ashby. 1999. Safety Evaluation of Phytosterol Esters. Part 1. Assessment of oestrogenicity using a combination of *in vivo* and *in vitro* assays. *Food Chem. Toxicol.* 37:13–22.

Balsam, M. S., and E. Sagarin. eds. 1974. *Cosmetics: Science and technology*, Vol. 3, 582–584. New York: John Wiley & Sons.

Biosearch Incorporated. 1987. Schwartz-Peck Prophetic Patch Test and Draize-Shelanski Repeated Insult Patch Test Conducted With Liquid Eyeliner 2506–130. Unpublished data submitted by Cosmetic, Toiletry, and Fragrance Association (CTFA), 5-1-97 (27 pages).[2]

Biosearch Incorporated. 1988. Cosmetic Product Usage Study. Unpublished data submitted by CTFA, 5-1-97 (431 pages).[2]

Biosearch Incorporated. 1991. Draize-Shelanski Repeated Insult Patch Test: SPF Foundation 3038-203 (3079-45). Unpublished data submitted by CTFA, 5-1-97 (14 pages).[2]

Biosearch Incorporated. 1992a. Irritation Screening Study. Unpublished data submitted by CTFA, 5-1-97 (12 pages).[2]

Biosearch Incorporated. 1992b. Modified Draize Repeated Insult Patch Test Study in Human Subjects. Unpublished data submitted by CTFA, 5-1-97 (11 pages).[2]

Biosearch Incorporated. 1992c. Human Phototoxicity Test. Unpublished data submitted by CTFA, 5-1-97 (8 pages).[2]

Biosearch Incorporated. 1992d. Human Photoallergy Test. Unpublished data submitted by CTFA, 5-1-97 (9 pages).[2]

Boberg, K. M., K. Einarsson, and I. Björkhem. 1990a. Apparent lack of conversion of sitosterol into C_{24}-bile acids in humans. *J. Lipid Res.* 31:1083–1088.

Boberg, K. M., E. Lund, J. Ölund, and I. Björkhem. 1990b. Formation of C_{21} bile acids from plant sterols in the rat. *J. Biol. Chem.* 265:7967–7975.

Boberg, K. M., K. S. Pettersen, and H. Prydz. 1991. Toxicity of sitosterol to human umbilical vein endothelial cells *in vitro*. *Scand. J. Clin. Lab. Invest.* 51:509–516.

Brock, W. 2000. Phytosterols and Phytosterol Esters: Toxicity Review. Unpublished data submitted by the Cosmetic, Toiletry, and Fragrance Association. 15 pages.[2]

Budavari, S., ed. 1989. *The Merck Index. An encyclopedia of chemicals, drugs, and biologicals*, 11th ed., 261, 1354, 1388. Rahray, NJ: Merck & Co., Inc.

Burck, P. J., A. L. Thakkar, and R. E. Zimmerman. 1982. Antifertility action of a sterol sulphate in the rabbit. *J. Reprod. Fert.* 66:109–112.

Chiang, H. C., T. H. Tseng, C. J. Wang, C. F. Chen, and W. S. Kan. 1991. Experimental antitumor agents from *Solanum indicum* L. *Anticancer Res.* 11:1911–1918.

Clayton, P. T., A. Bowron, K. A. Mills, et al. 1993. Phytosterolemia in children with parenteral nutrition-associated cholestatic liver disease. *Gastroenterology* 105:1806–1813.

Cosmetic Ingredient Review (CIR). 1996. Special Report on the Reproductive and Developmental Toxicity of Ethylene Glycol and its Ethers. Washington, DC: CIR.[2]

CIR. 1997. *Final Report on the safety assessment of PEG-5, -10, -16, -25, -30, and -40 Soy Sterol.* Washington, DC: CIR.

Cosmetic, Toiletry, and Fragrance Association (CTFA). 1997. PEG-5 and -25 Soy Sterol: Safety data summaries, 5-1-97 (2 pages).[2]

CTFA. (2000) Concentration of use of PEGs Soy Sterol in cosmetic products. Unpublished data submitted by CTFA (1 page).[2]

Covance Laboratories Ltd. 1999a. Plant Sterol Ester SSE26698-02: Measurement of Unscheduled DNA Synthesis in Rat Liver using an *in vivo/in vitro* Procedure. Study KU990111. Unpublished data submitted by CTFA. 37 pages.[2]

Covance Laboratories Ltd. 1999b. Plant Sterol Ester SSE26698-02: Induction of Micronuclei in the Bone Marrow of Treated Rats. Study KC990110. Unpublished data submitted by CTFA. 32 pages[2]

Deschner, E. E., B. I. Cohen, and R. F. Raicht. 1982. The kinetics of the protective effect of β-sitosterol against MNU-induced colonic neoplasia. *J. Cancer Res. Clin. Oncol.* 103:49–54.

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

Educational & Research Foundation, Inc. 1991a. 49-Day Usage Study in Human Subjects-Acnegenicity. Final Report: Liquid Make-Up With Sunscreen Formulas #3079-11 and #3079-13. Unpublished data submitted by CTFA, 5-1-97 (352 pages).[2]

Educational & Research Foundation, Inc. 1991b. 45-Day Usage Study in Human Subjects-Acnegenicity. Final Report: Liquid Make-Up Foundation #2978-188. Unpublished data submitted by CTFA, 5-1-97 (329 pages).[2]

Elder, R.L., ed. 1983. Final report on the safety assessment of PEG-2, -6, -8, -12, -20, -32, -40, -50, -100, and -150 Stearates. *J. Am. Coll. Toxicol.* 2:17–34.

Environmental Protection Agency (EPA). 2002. 40CFR180.1001. Tolerances and exemptions from tolerances for pesticide chemicals in food; subpart D—Exemptions from tolerances.

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

FDA. 1996. Frequency of use of cosmetic ingredients. *FDA database*. Washington, DC: FDA.

General Mills, Inc. 1979. Plant sterols, phytosterols, or sitosterols which are comprised of a mixture of the common sterol components. Submitted by FDA in response to an FOI request dated 3-15-96.[2]

Gould, R. G. 1955. Absorbability of beta-sitosterols. *Trans. N. Y. Acad. Sci.* 18:129–134.

Gould, R. G., R. J. Jones, G. V. LeRoy, R. W. Wissler, and C. B. Taylor. 1969. Absorbability of β-sitosterol in humans. *Metabolism* 18:652–662.

Hamburger, R., E. Azaz, and M. Donbrow. 1975. Autoxidation of polyoxyethylenic non-ionic surfactants and of polyethylene glycols. *Pharm. Acta Helv.* 50:10–17.

Heinemann, T., G. Axtmann, and K. von Bergmann. 1993. Comparison of intestinal absorption of cholesterol with different plant sterols in man. *Eur. J. Clin. Invest.* 23:827–831.

Heinemann, T., G. A. Kullak-Ublick, B. Pietruck, and K. von Bergmann. 1991. Mechanisms of action of plant sterols on inhibition of cholesterol absorption. *Eur. J. Clin. Invest.* 40(Suppl. 1):S59–S63.

Henkel Corp. 1995. Data on the PEGs Soya Sterol. Unpublished data submitted by CTFA, 4-22-96 (5 pages).[2]

Hennessey, T. M. 1992. Effects of membrane plant sterols on excitable cell functions. *Comp. Biochem. Physiol.* 101C:1–8.

Hepburn, P. A., S. A. Horner, and M. Smith. 1999. PA. Safety Evaluation of Phytosterol Esters. Part 2. Subchronic 90-day Oral Toxicity Study on Phytosterol Esters—A Novel Functional Food. *Food Chem. Toxicol*. 37:521–532.

Hirano, T., M. Homma, and K. Oka. 1994. Effects of stinging nettle root extracts and their steroidal components on the Na^+, K^+-ATPase of the benign prostatic hyperplasia. *Planta Med.* 60:30–33.

Hoch-Ligeti, C., M. F. Argus, and J. C. Arcos. 1970. Induction of carcinomas in the nasal cavity of rats by dioxane. *Br. J. Cancer* 24:164–167.

Huntingdon Life Sciences Ltd. 1996a. Phytosterol Esters: Bacterial Mutation Assay. Study KA960256. Unpublished data submitted by CTFA. 23 pages.[2]

Huntingdon Life Sciences Ltd. 1996b. Phytosterols: Bacterial Mutation Assay. Study KA960254. Unpublished data submitted by CTFA. 23 pages.[2]

Huntingdon Life Sciences Ltd. 1997a. Phytosterols: Metaphase Chromosome Analysis of Human Lymphocytes Cultured in vitro. Study KC960255. Unpublished data submitted by CTFA. 32 pages.[2]

Huntingdon Life Sciences Ltd. 1997b. Phytosterol Esters: Metaphase Chromosome Analysis of Human Lymphocytes Cultured in vitro. Study KC960257. Unpublished data submitted by CTFA. 34 pages.[2]

Huntingdon Life Sciences Ltd. 1998. Plant Sterols: Bacterial Mutation Assay. Study KA980008. Unpublished data submitted by CTFA. 26 pages.[2]

Janezic, S. A., and A. V. Rao. 1992. Dose-dependent effects of dietary phytosterol on epithelial cell proliferation of the murine colon. *Food Chem. Toxicol.* 30:611–616.

Kallianos, A. G., F. A. Shelburne, R. E. Means, R. K. Stevens, R. E. Lax, and J. D. Mold. 1963. Indentication of the D-glucosides of stigmasterol, sitosterol, and campesterol in tobacco and cigarette smoke. *Biochem. J.* 87:596–600.

Kociba, R. J., S. B. McCollister, C. Park, T. R. Torkelson, and P. J. Gehring. 1974. 1,4-Dioxane. I. Results of a 2-year ingestion study in rats. *Toxicol. Appl. Pharmacol.* 30:275–286.

Lange, W. 1950. Cholesterol, phytosterol, and tocopherol content of food products and animal tissues. *J. Am. Oil Chem. Soc*. 27:414–422.

Laraki, L., X. Pelletier, J. Mourot, and G. Debry. 1993. Effects of dietary phytosterols on liver lipids and lipid metabolism enzymes. *Ann. Nutr. Metab.* 37:129–133.

Lewis, R. J., Sr., ed. 1997. *Hawley's condensed chemical dictionary*, 1002. New York: John Wiley & Sons.

Ling, H. C., M. L. Ling, M. H. Su, G. L. Chen, and C. T. Wang. 1981. Study on antitumor plant *Gymnosporia trilocularis*. *J. Chin. Chem. Soc.* 28:95–101.

Ling, W. H., and P. J. H. Jones. 1995. Dietary phytosterols: A review of metabolism, benefits, and side effects. *Life Sci.* 57:195–206.

Lundmark, L., H. Chun, and A. Melby. 1976. Soya Sterols: Functional Plant-Derived Ingredients for Toiletries—Part 1. *Soap Cosmet. Chem. Spec*. 52: 33–4, 38, 40.

Lundmark, L., H. Chun, and A. Melby. 1977. Soya Sterols: Functional Plant-Derived Ingredients for Toiletries—Part II. *Soap Cosmet. Chem. Spec.* 53:33–4, 36, 66.

Malini, T., and G. Vanithakumari. 1990. Rat toxicity studies with β-sitosterol. *J. Ethnopharma.* 28:221–234.

Malini, T., and G. Vanithakumari. 1991. Antifertility effects of β-sitosterol in male albino rats. *J. Ethnopharma.* 35:149–153.

Malini, T., and G. Vanithakumari. 1992. Comparative study of the effects of β-sitosterol, estradiol and progesterone on selected biochemical parameters of the uterus of ovariectomised rats. *J. Ethnopharma.* 36:51–55.

Malini, T., and G. Vanithakumari. 1993. Effect of beta-sitosterol on uterine biochemistry: A comparative study with estradiol and progesterone. *Biochem. Mol. Biol. Int.* 31:659–668.

McGinity, J. W., J. A. Hill, and A. L. La Via. 1975. Influence of peroxide impurities in polyethylene glycols on drug stability. *J. Pharm. Sci.* 64:356–357.

Mellanen, P., T. Petänen, J. Lehtimäki, S. Mäkelä, G. Bylund, B. Holmbom, E. Mannila, A. Oikari, and R. Santti. 1996. Wood-derived estrogens: Studies in vitro with breast cancer cell lines and in vivo in trout. *Toxicol. Appl. Pharmacol*. 136:383–388.

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

North American Science Associated, Inc. 1987a. Acute Dermal Toxicity Study in the Rabbit of Liquid Eyeliner Formula No. 2506-130. Unpublished data submitted by CTFA, 5-1-97 (6 pages).[2]

North American Science Associated, Inc. 1987b. Primary Skin Irritation Test in the Rabbit of Liquid Eyeliner Formula No. 2506-130. Unpublished data submitted by CTFA, 5-1-97 (6 pages).[2]

North American Science Associated, Inc. 1987c. Ocular Irritation Test in the Rabbit of Liquid Eyeliner Formula No. 2506-130. Unpublished data submitted by CTFA, 5-1-97 (8 pages).[2]

North Cliff Consultants, Inc. 1992. Home Use Study of a Mascara Product to Determine the Potential for Irritation. Unpublished data submitted by CTFA, 5-1-97 (271 pages).[2]

Padmaja, V., V. Thankamany, and A. Hisham. 1993. Antibacterial, antifungal, and anthelmintic activities of root barks of *Uvaria hookeri* and *Uvaria narum*. *J. Ethnopharma.* 40:181–186.

Pepe, R. C., J. A. Wenninger, and G. N. McEwen, Jr., eds. 2002. *International cosmetic ingredient dictionary and handbook*, 9th ed. Washington, DC: CTFA.

Peterson, D. W. 1951. Effet of soybean sterols in the diet on plasma and liver cholesterol in chicks. *Proc. Soc. Exp. Biol. Med.* 78:143–147.

Pollak, O.J. 1953. Reduction of blood cholesterol in man. *Circulation* 7:702–706.

Pollak, O. J. 1985. Effect of plant sterols on serum lipids and atheroschlerosis. *Pharmac. Ther.* 31:177–208.

Raicht, R. F., B. I. Cohen, E. P. Fazzini, A. N. Sarwal, and M. Takahashi. 1980. Protective effect of plant sterols against chemically induced color tumors in rats. *Cancer Res.* 40:403–405.

Robinson, J. J., and E. W. Ciurczak. 1980. Direct gas chromatographic determination of 1,4-dioxane in ethoxylated surfactants. *J. Soc. Cosmet. Chem.* 31:329–337.

Rosenblum, E. R., R. E. Stauber, D. H. Van Thiel, I. M. Campbell, and J. S. Gavaler. 1993. Assessment of the oestrogenic activity of phytoestrogens isolated from bourbon beer. *Alcoholism Clin. Exp. Res*. 17:1207–1209.

Rothschild, D. L., Jr. 1990. *The Food Chemical News Guide to the Current Status of Food and Color Additives*, 340–345. Washington, DC: Food Chemical News, Inc.

Sabine, J. R. 1977. *Cholesterol*. New York: Marcel Dekker, Inc.

Saeed, S. A., S. Farnaz, R. U. Simjee, and A. Malik. 1993. Triterpenes and β-sitosterol from piper betle: isolation, antiplatelet, and anti-inflammatory effects. *Biochem. Soc. Trans.* 21:462S.

Sanders, D. J., H. J. Minter, D. Howes, and P. A. Hepburn. 2000. Safety evaluation of phytosterol esters. Part 6. The comparative absorption and tissue distribution of phytosterols in the rat. *Food Chem. Toxicol*. 38:485–491.

Sato, Y., K. Nishikawa, K. Aikawa, et al. 1995. Side-chain structure is critical for the transport of sterols from lysosomes to cytoplasm. *Biochim. Biophys. Acta.* 1257:38–46.

Shipley, R. E., R. R. Pfeiffer, M. M. Marsh, and R. C. Anderson. 1958. Sitosterol feeding: Chronic animal and clinical toxicity and tissue analysis. *Circ. Res.* 6:373–382.

Silverstein, B. D., P. S. Furcinitti, W. A. Cameron, J. E. Brower, and O. White, Jr. 1984. Biological effects summary report—polyethylene glycol. Government Reports Announcements & Index. Issue 15. NTIS No. DE84007984.

Swern, D. ed. 1979. *Bailey's industrial oil and fat products*, Vol. 1, 55, 62. New York: John Wiley & Sons.

Tso, P., and K. Fujimoto. 1991. The absorption and transport of lipids by the small intestine. *Brain Res. Bull.* 27:477–482.

Tvrzická, E., P. Mares, A. Písaríková, J. Novakovic, and P. Hrabák. 1991. Simplified gas chromatographic method for the simultaneous determination of phytosterols and cholesterol. *J. Chromatogr.* 563:188–192.

Tyle, P., and S. G. Frank. 1990. Phytosterol stabilized emulsions: Interfacial complexation and structural investigations. *Drug Dev. Ind. Pharm.* 16:1605–1618.

Tyle, P., and S. G. Frank. 1991. Penetration temperatures of aqueous sodium lauriminodipropionate solutions into solid phytosterols. *J. Pharm. Sci.* 80: 201.

Unilever. 1996a. Phytosterols (Ex Roche): Chemical Characterization. Study AC960273. Unpublished data submitted by CTFA. 24 pages.[2]

Unilever. 1996b. Phytosterol Esters (Ex Roche): Chemical Characterization. Study AC960274. Unpublished data submitted by CTFA. 25 pages.[2]

Unilever. 1997a. The Fate in the Male Rat of [^{14}C]β-sitosterol and [^{14}C]β-sitosterol Linoleate following Gavage Administration. Study AM970460. Unpublished data submitted by CTFA. 51 pages.[2]

Unilever. 1997b. [^{14}C]β-sitosterol and [^{14}C]β-sitosterol Linoleate: The Distribution and Metabolism in the Rat following Gavage Administration. Study AM970152. Unpublished data submitted by CTFA. 64 pages.[2]

Unilever. 1998a. Plant Sterols: Characterization and Stability. Study AC960458. Unpublished data submitted by CTFA. 22 pages.[2]

Unilever. 1998b. Plant Sterols: Characterization and Stability. Study AC970209. Unpublished data submitted by CTFA. 24 pages.[2]

Vanhanen, H. T., and T. A. Miettinen. 1992. Effects of unsaturated and saturated dietary plant sterols on their serum contents. *Clin. Chim. Acta* 205:97–107.

Waalkens-Berendsen, D. H., A. P. M. Wolterbeek, M. V. W. Wijnands, M. Richold, and P. A. Hepburn. 1999. Safety evaluation of phytosterol esters. Part 3. Two-generation reproduction study in rats with phytosterol esters—a novel functional food. *Food Chem. Toxicol*. 37:683–696.

Warf Institute, Inc. 1974. Oral LD50, skin irritation, and eye irritation of the PEGs Soy Sterol. (Henkel Corp.) Unpublished data submitted by CTFA, 4-22-96. 16 pages.

Yasukawa, K., M. Takido, T. Matsumoto, M. Takeuchi, and S. Nakagawa. 1991. Sterol and triterpene derivatives from plants inhibit the effects of a tumor promoter, and sitosterol and betulinic acid inhibit tumor formation in mouse skin two-stage carcinogenesis. *Oncology* 48:72–76.

Zava, D. T., C. M. Dollbaum, and M. Blen. 1998. Estrogen and pregestin bioactivity of foods, herbs, and spices. *Proc. Soc. Exp. Biol. Med*. 217:369–378.