Safety Assessment of Phytosterols as Used in Cosmetics

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All interested persons are provided 60 days from the above date to comment on this Amended Tentative Safety Assessment and to identify additional published data that should be included or provide unpublished data which can be made public and included. Information may be submitted without identifying the source or the trade name of the cosmetic product containing the ingredient. All unpublished data submitted to CIR will be discussed in open meetings, will be available at the CIR office for review by any interested party and may be cited in a peer-reviewed scientific journal. Please submit data, comments, or requests to the CIR Director, Dr. Lillian J. Gill.

The 2013 Cosmetic Ingredient Review Expert Panel members are: Chairman, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; Ronald A Hill, Ph.D. James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is Lillian J. Gill, D.P.A. This report was prepared by Lillian C. Becker, Scientific Analyst/Writer.

1101 17th Street, NW, Suite 412 ♢ Washington, DC 20036-4702 ♢ ph 202.331.0651 ♢ fax 202.331.0088 ♢ cirinfo@cir-safety.org

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ABSTRACT

Phytosterols function in cosmetics include skin-conditioning agents, hair conditioning agents, viscosity increasing agents, skin protectants, antioxidants, and fragrances. The Panel reviewed relevant animal and human data related to these ingredients including results of tests for estrogenic effects. The Panel concluded that phytosterols were safe as cosmetic ingredients in the practices of use and concentration of this safety assessment. Industry should use good manufacturing practices to limit impurities.

INTRODUCTION

This report reviews the available scientific information relevant to the safety of a group of 26 phytosterols and steryl alkanoates as used in cosmetics. The functions of these ingredients include: skin-conditioning agents, hair conditioning agents, viscosity increasing agents, skin protectants, antioxidants, and fragrances (Table 1).[1] The ingredients in this report are:

- brassica campestris (rapeseed) sterols
- canola sterols
- C10-40 isoalkyl acid phytosterol esters
- dihydrophytosteryl octyldecanoate
- euterpe oleracea sterols
- glycine soja (soybean) sterols
- persea gratissima (avocado) sterols
- phytosterols
- phytosteryl butyrate
- phytosteryl canolate
- phytosteryl caprylate/caprate
- phytosteryl hydroxystearate
- phytosteryl isostearate
- phytosteryl linoleate
- phytosteryl linoleate/linolenate
- phytosteryl macadamiate
- phytosteryl nonanoate
- phytosteryl oleate
- phytosteryl rice branate
- phytosteryl ricinoleate
- phytosteryl sunflowerseedate
- punica granatum sterols
- beta-sitosterol
- beta-sitosteryl acetate
- soy sterol acetate
- tall oil sterol

Plant sterols, or phytosterols, occur naturally as free alcohols and as fatty acid esters (i.e., naturally occurring steryl alkanoates). The ingredients in this report are sterol alcohols or esters (in some cases mixtures of both) extracted from plants, which in some cases have been saponified to the free alcohols and then esterified with plant-derived fatty acids. The resultant ester-derivatized phytosterols (i.e., steryl alkanoates) share substantial component overlap with the naturally-occurring phytosterol esters. Most of these derived esters are synthetic copies of the components of the naturally occurring phytosterol esters. Because there is expected to be a large amount of component overlap among the ingredients in this group, these ingredients are amenable to reviewing them as an ingredient family and employing read-across. The structural similarities among the compounds or components (i.e., phytosterols and fatty acid), the similarities of the physicochemical properties of same, and functions and concentrations of these ingredients in cosmetics enable grouping these ingredients and reading across the available toxicological data to support the safety assessment of the entire group. Table 2 lists the component compounds, noting whether or not these components are cosmetic ingredients, whether or not they have been reviewed by the Cosmetic Ingredient Review (CIR) Expert Panel (Panel), and, if so, the Panel's conclusions. All of the reviewed component cosmetic ingredients were found to be safe as used. Butyric acid, caprylic acid/capric acid, and linoleic acid/linolenic acid have not been reviewed. Octyldecanoic acid is not a cosmetic ingredient.

In 2000, the Panel found the data on PEG-5, -10, -16, -25, -30, and -40 soy sterols to be insufficient to support the safety of these ingrdients.[2] In 2004, the Panel found these PEG soy sterols to be safe as used in an amended safety assessment that included data on phytosterols and phytosterol esters.[3] The Panel's approach in these safety assessments was to review the safety of PEGs and phytosterols/soy sterols, as well as the conjugated polyethers, and assess the safety of the PEG phytosterols from those data. Because the data on the phytosterols/soy sterols are relevant for this safety assessment, summaries of the phytosterols/soy sterols data from these two safety assessments are provided in the appropriate sections.

Many of the phytosterols in this study are from edible plant sources. Exposure to these phytosterols from consuming foods results in much greater systemic doses than could result from the use of cosmetic products. It was noted in the PEG soy sterol reports that phytosterols and phytosterol esters are not significantly absorbed after oral exposure, and thus, did not result in systemic exposure.[2,3] Therefore, acute and repeated dose oral toxicity potential of these phytosterols as cosmetic ingredients will not be addressed again in depth in this report. The focus of this report is on: reproductive toxicity, genotoxicity, carcinogenicity, dermal irritation, and sensitization. Pertinent data from the PEG soy sterol safety assessment are summarized below in the appropriate sections.

CHEMISTRY

Definition, Structure, and Composition

See Table 1 for definitions and function information.

The phytosterol ingredient group is comprised of the plant-derived free sterols and their esters, the steryl alkanoates. β-Sitosterol is an example of a discreet, free phytosterol ingredient.

To generate a steryl alkanoate with an ester at the 3-position of the sterol, the hydroxyl group at the 3-position of the cyclopentenophenanthrene scaffold is esterified by reacting with an alkyl acid or acid chloride (i.e., β-Sitosteryl Acetate). Representative phytosterols are also provided in Figure 1.

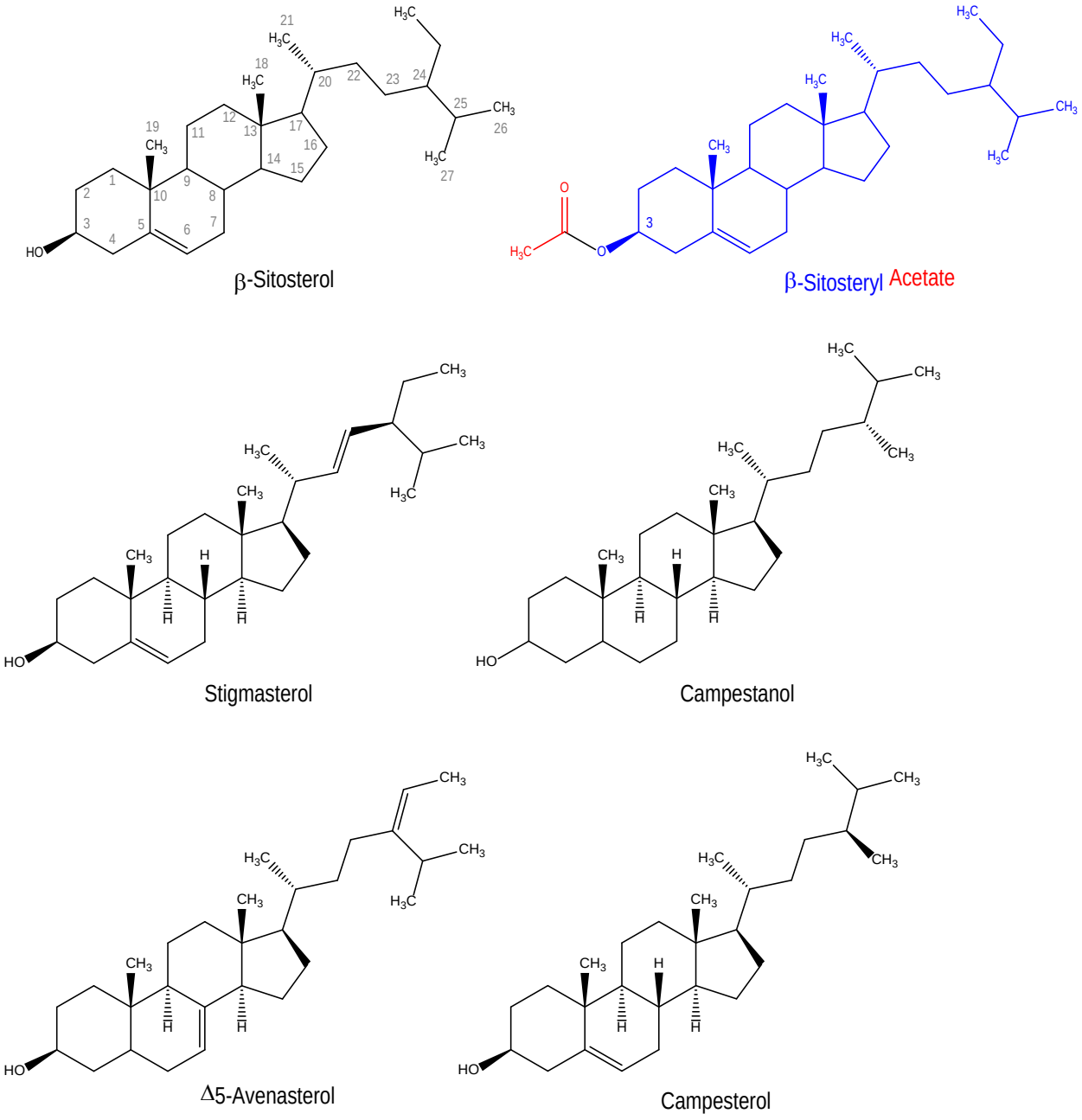

Figure 1. Examples of discreet molecules found in phytosterol ingredients

Phytosterols occur in plants in the free alcohol, steryl alkanoate, or glycoside forms. The free phytosterols are characteristic components of the non-saponifiable fractions of plant oils.[4] The steryl alkanoate and glycoside forms,

however, are broken down to the free phytosterol form (and respective acid or sugar) under saponification conditions. The majority of the ingredients in this report are mixtures of either sterols or steryl alkanoates, with component concentrations that vary with growth and extraction conditions.

As one example, soybean oil that had been alkali-refined typically contained 0.446 mg/100 mg oil of total sterols and 0.287 mg/100 mg oil of free sterol. The ratio of esterified to free sterol was 0.55.[5]

Palm oil/crude palm oil contains 300 – 620 ppm phytosterols, 60% of which is beta-sitosterol with 38% being stigmasterol and campesterol.[6]

Product information on refined soy sterols reported that they contain ~ 88% total sterol content. Of that percentage, 56% is γ-sitosterol, 28% is campesterol, and 4% is stigmasterol. Other compounds isolated from the phytosterols are 4% - 6% sterol hydrocarbons and cholesterol, and 4% - 6% triterpene alcohols, keto-steroids, and other steroid-like substances.[7]

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The chemical characterization of a plant sterol material is provided in Table 3. The distributions of phytosterols in common vegetable oils are provided in Table 4.

In an analysis of another source of phytosterols (source not provided), it was reported that the principal phytosterols were present as follows: β-sitosterol, 47.9%; campesterol, 28.8%; and stigmasterol, 23.3%. In an analysis of phytosterol esters, it was 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.[8,9]

Physical and Chemical Properties

Physical and chemical properties of representative phytosterols in the form of vegetable oil sterols and tall oil sterols are provided in Table 5.

Phytosterols and their fatty acid esters are thermally stable and degrade only at high temperatures (>100°C) in the presence of oxygen.[10]

A typically palm phytosterol is characterized as a crystaline waxy powder or a free-flowing granular powder that is white to off white and practically odorless.[6,11] Its melting point is reported to be 131-141°C. The phytosterol content is at least 80% (50% beta sitosterol; 20% campesterol; and 2% stigmasterol). Palm-derived phytosterols were reported to be stable for 36 months when stored in a cool dry environment in unopened containers. The product is sensitive to air, light, and heat.

Method of Manufacture

Free phytosterol alcohols and phytosterol alkanoates are characteristic components of plant oils; saponification of these oils is the primary means of producing free phytosterol alcohols for commercial use.[4]

Soy sterol is isolated from soybean oil distillates in a saponification process in which the phytosterol alcohols are separated from the fatty acids by extraction with a fat solvent.[2] The phytosterols in the resulting extract are separated from the tocopherols in the mother liquor, and then purified and/or separated into constituent sterols.

Tall oil sterol, an example of a phytosterol mixture, is obtained from tall oil soap in a multi-step process.[4] The production process involves fractional distillation of the tall oil soap to remove volatile compounds. The resulting residue (tall oil pitch), containing esterified sterols (i.e., steryl alkanoates), is treated with alkali (saponified) to release the free sterol alcohols. After neutralization, the material is subjected to a two-stage distillation process. The distillate is then dissolved in methanol/methylethylketone solvent and the sterols crystallizing from this solution are obtained by filtration, washed with solvent and dried. This procedure results in a lower stanol and a higher sterol content of the phytosterol mixture. Conifers that have naturally lower stanol content are now used as the primary source of the tall oil soap. Stanols (obtained by catalytic hydrogenation of the phytosterol mixture) are added before the crystallization step to maintain the original stanol/sterol ratio. The phytosterol composition of the tall oils produced from the two processes is provided in Table 6.

Steryl alkanoates are produced from free sterols by classical esterification methods, using acids or acid chlorides. Sterol alkanoates may be derived from neutralized, refined, bleached and deodorized (N/RBD) reaction with soybean distillates.[12,13] Crude soybean oil is degummed, neutralized, bleached and deodorized to yield N/RBD soybean oil and distillates. The deodorized distillate undergoes further processing (crystallization and/or distillation), resulting in a sterol mixture. This sterol mixture is then crystallized and esterified with fatty acids (from food grade vegetable oils such as rapeseed or sunflower oil), washed, bleached and deodorized to give the final plant steryl alkanoates.

A manufacturer reported that for the manufacture of glycine soja (soybean) sterols, and punica granatum sterols, the raw materials are tested for acceptable qualifications (not specified) before they are cold pressed for oil.[14,15] The oil is then tested for quality (not specified) before the oil is fractionated to isolate the sterols. Pomegranate sterols are heat-sterilized at 100°C before fractionation.[16]

A manufacturer reports that the extraction process for palm oil phytonutrients is an integrated process for the recovery of phytosterols as well as vitamin E and squalene.[11] The process comprises the steps of acid/alkaline catalyzed esterification/transesterification process of palm oil with a lower alkyl alcohol, multi-stage vacuum distillation of alkyl esters,

saponification of the phytonutrients concentrate, crystallization of phytosterols, and partitioning of vitamin E and squalene with organic solvents.

Impurities

In assessing the data on soybean oil sterols, the Scientific Panel on Dietetic Products, Nutrition and Allergies noted that there are limited analytical data of sufficient sensitivity and reliability regarding the possible residual allergen (protein) content of phytosterols.[12] Specifically, the limited analytical data regarding the protein (allergen) content of N/RBD soybean oil-derived plant stanol esters were insufficient to predict the likelihood of adverse reactions in soybean-allergic individuals. This Panel concluded, however, that because the starting material is refined soybean oil and there is an adequate subsequent production process, it is not very likely that this product will retain enough residual protein to cause a severe allergic reaction in the majority of soybean-allergic individuals.

The final protein content of N/RBD soybean oils (the source of soy phytosterols) is known to depend on the quality and efficiency of purification steps.[12] The protein content of N/RBD oils may be reduced to low levels within the 0.02-0.44 μg/kg range.[17] When two samples of edible soy oil (crude virgin and deodorized) were analyzed for proteins, 1.89 μg/mL and 0.32 μg/mL proteins were found in the samples. [18]

When selected phytosterol samples (a phytosterol blend and a phytosterol blend spiked with reference protein) were analyzed for residual soybean protein using ELISA (enzyme-linked immunosorbent assay), soy protein was not detectable at or above the 10-20 μg/g detection limit.[19]

Commercial tall oil sterols/stanols were reported to contain < 0.1 mg/kg lead.[10] Commercial vegetable oil sterols, in general, were reported to have < 2.0 mg/kg impurities (mercury, < 0.1%; lead, < 0.1%; cadmium, < 0.1%; and arsenic, < 0.1%). Both were reported to contain < 2 ppb PAHs and < 1.5 ng-TEQ/kg dioxins and dioxin-like PCBs. No pesticides were detected.

In an analysis of *Euterpe oleracea* sterols, glycine soja (soybean) sterols, and *Punica granatum* sterols, none of these ingredients contained detectable levels of an array of potential allergens, including amyl cinnamal, benzyl alcohol, citronellol, coumarin, linalool, and farnesol (Table 7). Another analysis did not detect any of several pesticides, including DDT (detection level 1.00 mg/kg), methidathion (0.20 mg/kg), and pyrethrins (3.00 mg/kg).[20-22]

Phytosterols derived from palm phytosterol are reported to typically meet the following parameters: mercury, < 1 ppm; cadmium, < 1 ppm; arsenic, < 1 ppm; lead, < 1 ppm; benzo(a)pyrene, < 2 ppm.[11] They are allergen free with regards to peanut, eggs, milk/milk by-products, wheat or gluten, tree nuts, soy, fish and shellfish. Assays for pesticide impurities are reported as follows: araquat, <0.05 ppm; diquat, <0.05 ppm; DDT, <0.05 ppm, hexachlorocyclohexane, <0.05 ppm. These pesticides are known to be used in palm oil plantations in Malaysia.

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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.[23] In an analysis of phytosterols (source not provided), no impurities were found. [8,9]

USE

Cosmetic

Data on ingredient usage are provided to the Food and Drug Administration (FDA) Voluntary Cosmetic Registration Program (VCRP; Table 8).[24] A survey was conducted by the Personal Care Products Council (Council) of the maximum use concentrations for ingredients in this group.[25]

Data were available from both the VCRP and the Council for the following ingredients:

- Brassica campestris (rapeseed) sterols was reported to be used in 50 leave-on products and 7 rinse-off products. Leave-on products were reported to contain up to 7% (the highest amount in lipstick) and 0.13% in rinse-off products.
- Glycine soja (soybean) sterols was reported to be used in 194 leave-on products (mostly skin care and makeup products), 45 rinse-off products, and one bath product. Leave-on products were reported to contain up to 1% (the highest concentration in eye lotion, cuticle softeners, and other skin preparations) and up to 4.1% in rinse-off products (the highest concentration in skin cleansing products). This ingredient was reported to be used in tonics, dressings and other hair grooming aids, including an aerosol and a pump spray at 0.000001%.
- Phytosterols was reported to be used in 177 leave-on products. It was reported that phytosterols was used in lipsticks up to 5%, non-spray deodorants up to 0.06%, and eye makeup up to 2%. It is also used in 215 rinse-off products. It was reported that phytosterols was used in hair products up to 2.4%, bath soaps and detergents up to 0.005%, and indoor tanning preparations up to 0.0001%. It was also reported to be used in face powders up to 0.05%.
- Phytosteryl isostearate was reported to be used in 15 leave-on products one rinse-off product. This ingredient was reported to be used up to 3% in leave-on products and in one rinse-off product up to 0.5%. It is used in lipsticks up to 3% and in eye makeup up to 0.5%.

- Phytosteryl [phytosterol] macadamiate was reported to be used in 181 leave-on products (100 lipsticks) and in two rinse-off products. It was reported to be used in leave-on products up to 8% and in rinse-off products up to 1%. It was reported to be used in lipsticks up to 7% and in moisturizing products up to 8%.
- Phytosteryl oleate was reported to be used in 20 leave-on products (including 6 paste masks/mud packs). It was reported to be used up to 3%.
- Phytosteryl rice branate was reported to be used in an eye makeup and a moisturizing product. The Council reported that it was used in eye lotions up to 1%, foundations up to 0.5%, and face and neck products up to 0.5%.
- Punica granatum sterols was reported to be used in 29 rinse-off products and in two rinse-off products. It was reported to be used up to 5% in leave-on products (including lipsticks).
- Beta-sitosteryl was reported to be used in 46 leave-on products and in two rinse-off products. It was reported to be used in leave-on products up to 0.06%.
- Tall oil sterol was reported to be used in 7 leave-on products. It was reported to be used up to 0.0046%, including in skin cleansing products up to 0.0006%.

Data were available only on the frequencies of use (VCRP) for the following ingredients:
- Euterpe oleracea sterols was reported to be used in one lipstick and one foundation.
- Soy sterol acetate was reported to be used in one moisturizing product.

Data were available only on use concentrations (Council) for the following ingredients:
- Persea gratissima (avocado) sterols was reported to be used in eye lotion up to 1%, lipstick up to 0.65%, and face and neck products up to 0.1%.
- Phytosteryl canolate was reported to be used in eye shadow up to 0.06%.

There were no use or concentration of use data reported for:

- Canola sterols
- C10-40 isoalkyl acid phytosterol esters
- Dihydrophytosteryl octyldecanoate
- Diosgenin
- Phytosteryl butyrate
- Phytosteryl caprylate/caprate
- Phytosteryl hydroxystearate
- Phytosteryl linoleate
- Phytosteryl linoleate/linolenate
- Phytosteryl nonanoate
- Phytosteryl ricinoleate
- Phytosteryl sunflowerseedate
- Punica granatum sterols
- Beta-sitosteryl acetate

As noted above, uses were reported for glycine soja (soybean) sterols in propellant and pump spray tonics, dressings and other hair grooming aids up to 0.000001%, and phytosterols are reported to be used in face powders up to 0.05%. In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters >10 µm, with propellant sprays yielding a greater fraction of droplets/particles below 10 µm compared with pump sprays. Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respired (i.e., they would not enter the lungs) to any appreciable extent.[26-31]

Non-Cosmetic

Phytosterols (stigmasterol-rich plant sterols: stigmasterol, >85%; brassicasterol, 1.7%; β-sitosterol, 3%; campesterol, 1.7%) are used in ready-to-freeze alcoholic beverages as a stabilizer.[32]

Phytosterols and phytostanols are commonly used in food products for their property of absorption reduction of cholesterol in the gut and lower cholesterol content by substituting for it in food products.[10] The optimal daily intake for the former purpose is 2 - 3 g. For example, in Europe, phytosterol esters are added to margarines and low-fat spreads (3.4 g/30 g), yogurts (1.25 g/125 mL), yogurt drinks (3.4 g/100mL), and milk (5 g/L).

Suggested uses for phytosterols from palm oil were: dietary supplements, functional ingredient for margarine/butter/oil, an emulsifier, and feed additives for prawns, lobsters, etc.

The Scientific Committee on Food (SCF) and European Food Safety Authority (EFSA) concluded that phytosterols, phytostanols and their esters could safely be approved for use in various foods (i.e., yellow fat spreads, soya drinks, salad dressings, rye bread) within the EU at levels resulting in intake of up to 3 g/day.[12,13,19,33-44]

TOXICOKINETICS

Absorption, Distribution, Metabolism, and Excretion

No published dermal or inhalation ADME studies were discovered and no unpublished data were submitted.

Oral

The Western diet consists of ~160-360 mg/d phytosterols consisting of ~80% β-sitosterol. The diet also includes some campesterol and stigmasterol, small amounts of brassicasterol, and trace amounts of Δ-5-saturated plant stanols.[45]

Less than 5% of dietary phytosterols, phytostanols, and their esters are absorbed in the gastrointestinal tracts of rats and humans.[32] Following absorption, phytosterols/phytostanols are transported in the serum via high density lipoproteins (HDL) in rats and low density lipoproteins (LDL) in humans to various organs and tissues, mostly to the liver. In the liver, phytosterols may be converted to bile acids. Absorbed phytosterols and phytostanols are predominantly excreted as such or as bile acids by the biliary route into the feces. The metabolic fate of phytosterols, phytostanols, and their esters is similar between rats and humans. The individual plant sterols are metabolized in a similar manner to each other. The phytosterols that are not absorbed in the gastrointestinal tract, or excreted as such in the bile, continue to the colon intact and are excreted in the feces.[45-48]

In an oral study (n = 10 healthy men), the average intestinal absorption of individual phytosterols were: campesterol, 9.6%; stigmasterol, 4.8%; and sitosterol, 4.2%.[49] The authors noted that these results were consistent with the results of animal studies showing that, for analogs of cholesterol, increasing the side-chain length of cholesterol reduced the absorbability of the sterol, with the exception of campesterol. The 5α-campesterol-saturated (the corresponding stanol) had greater absorbability than campesterol. Absorption was measured by an intestinal perfusion technique over a 50-cm segment of the upper jejunum.

In another study using male subjects, the biliary secretion rate of β-sitosterol was faster (1.23 mg/h) than that of campesterol (0.76 mg/h).[50]

Plant sterols, including stigmasterol and stanols (34 g/kg in feed), were able to cross the blood-brain barrier in a 90-day feeding study of Watanabe heritable hyperlipidemic rabbits.[51]

Cytotoxicity

β-sitosterol (200 µg/mL in ethanol) and β-sitosterol/campesterol (50%/40%; 200 µg/mL in ethanol) were cytotoxic to mouse macrophages (strain C57BL/6).[52] Cytotoxicity was demonstrated through cell viability, lipid uptake, lactate dehydrogenase (LDH) leakage, cellular protein content, and a 3'-[1-(phenylaminocarbonyl)-3,4-tetrazolium]bis(4-methoxy-6-nitro)benzene sulfonic acid hydrate (XTT) assay.

Phytosterols (0.01 – 40 mM; derived from pomegranate) were not cytotoxic in a Neutral Red Cytotoxicity assay.[53]

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β-Sitosterol (l00 µg/ml; 5% in DMSO and saline) was cytotoxic to seven cancer cell lines.[54]

ANIMAL TOXICOLOGY

Many of the phytosterols in this report are from edible sources; exposure to these phytosterols from food would presumably result in much larger systemic doses than those resulting from use in cosmetic products. A summary of toxicity data on phytosterols, including oral data, from the PEG soy sterol report is presented below. However, this present report does not focus on oral toxicity, but instead on the potential for reproductive toxicity, genotoxicity, carcinogenicity, irritation and sensitization via routes of exposure consistent with currently reported uses in cosmetics. A summary of toxicity data on phytosterols, including oral data, from the PEGylated soy sterol report is presented below for background information on toxicity.

Dermal - Non-Human

The dermal LD_{50} of two mixtures of phytosterol esters was reported to be > 2000 mg/kg.[55] A wood-derived mixture (a stanol composition ~94% β-sitostanol and ~6% campestanol in corn oil; WDPSE) and a vegetable oil–derived mixture of phytostanol esters (~68% β-sitostanol and ~32% campestanol in corn oil; VODPSE) were administered dermally to rats (n = 5/sex) for 24 h in accordance with to the Organization for Economic Co-operation and Development (OECD) Test Guideline 404. No deaths or clinical signs of toxicity were observed after application of WDPSE. One male rat in the VODPSE group died of unrelated causes during the 14-day observation period.

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Wistar rats administered a basal diet supplemented with cholesterol and maize phytosterols (72.5% β-sitosterol, 0.5% campesterol, and 7% stigmasterol) had decreased hepatic cholesterol concentrations.[56] Rats given the high dose of cholesterol and phytosterols had decreased malic enzyme and acetylCoA carboxylase activities, and had hypotriglyceridemia.

Wistar rats administered subcutaneous injections of 2.5 to 5 µg/1 g β-sitosterol for 60 days had no gross or microscopic lesions of the liver or kidneys.[57] Rats administered 10 µg/1 g had mild fibroblastic proliferation around the hepatic lobules and mild microscopic lesions of the kidney. Serum cholesterol was reduced in a dose-dependent manner, and serum protein was markedly reduced in rats of the high dose group.

In a 90-day oral toxicity study in female Wistar rats (n = 4), diets containing plant phytosterol esters up to 8.1 % were well tolerated.[58] Some small hematology and blood chemistry variations from the controls were observed. No

treatment related effects were observed with organ weights and histological examination and there was no evidence of systemic toxicity. Absent any organ effects, the small hematology and blood chemistry variations were not considered of toxicological significance.

Thirteen dogs fed a basic diet supplemented with 0.5 to 1.0 g/kg/day 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.[23]

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.[23]

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

In a two-generation feeding study, the no observed adverse effect level (NOAEL) for phytosterol esters was ≥8.1% (the highest dose tested) in the diet.[59,60] Wistar rats, F_0 generation, (n = 28/sex) were administered phytosterol esters (0, 1.6%, 3.2%, 8.1%) in feed for 10 weeks before mating, and continuing through gestation and weaning. The F_1 generation (n = 28/sex) were fed the same diet as their F_0 parents and mated after 10 weeks. The analysis of the phytosterols revealed the following breakdown: brassicasterol (2.9%), campesterol (26.7%), stigmasterol (17.7%), β-sitosterol (51.0%), cholesterol (0.2%), and unknowns (1.5%).

There were no maternal or teratogenic effects attributed to the test substance. There were no effects on fertility and reproductive parameters, including sexual maturity, estrous cycle length, precoital time, and the histopathology of reproductive tissues in either generation. There were no developmental or reproductive effects observed in either generation. Necropsies were unremarkable.

The NOAEL (8.1%) is equivalent to 3.3-6.5 g phytosterol esters/kg/d during the 10-week pre-mating period (~ 2.1-4.1 g phytosterols/kg/d or 400-900 mg stigmasterol/kg/d) and 2.5-9.1 g phytosterol esters/kg/d during gestation (~1.4-5.7 g/kg/d or 300-1200 mg stigmasterol/kg/d). The authors concluded that 2.5-9.1 g phytosterol esters/kg/d and 1.54-5.62 g phytosterols/kg/d (~ 335-1219 mg stigmasterol/kg/d), dependent on the phase of the study, was the NOAEL of daily oral administration of phytosterol esters for two successive generations.[59,60]

There were no signs of reproductive toxicity to American minks (n = 70/sex) orally administered β-sitosterol (at 0, 5, 10 or 50 mg/kg/d) for 10 months.[61] In the second part of the study, after 7 months of exposure, males (n = 10–11) were mated with 4–5 females each. There were no differences in number of pregnant females, litter and kit numbers, postnatal mortality and development and there were no treatment-related changes. After 3 months of exposure, 15 males/group were killed and investigated for organ weights and hematological and clinical chemistry parameters. Males exhibiting low quality fur were selected for this part of the study. There were differences in body fat masses (omental, mesenteric, retroperitoneal, intra-abdominal fat) reported, but increases in fat masses were not dose dependent. There were increased blood hemoglobin and serum high-density lipoprotein cholesterol concentrations observed.

Subcutaneous injections of β-sitosterol (5 mg/kg/d) for 16 to 48 days decreased sperm concentrations and fertility, and decreased testis and accessory sex tissue weights in a time-dependent manner in male Wistar rats.[62] Rats administered 0.5 mg/kg/d had a 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 termination of treatment, and appeared to be due to a reduction in the rate of spermatogenesis.

TESTS FOR ESTROGENIC EFFECTS

In Vitro

There were no signs of estrogenic activity of phytosterols and phytosterol esters in an in vitro competitive estrogen receptor binding assay (up to 1 x 10-4 mol/L) and a recombinant yeast assay (2×10^{-4} mol/L).[63] The phytosterols tested consisted of a mixture of β-sitosterol (47.9%), campesterol (28.8%), and stigmasterol (23.3%) and were sourced from a variety of edible vegetable oil distillates (e.g., sunflower, soya bean and rapeseed oils). The esters were phytosterols esterified with fatty acids from sunflower oil. The competitive estrogen receptor binding assay used a preparation of estrogen receptors isolated from 10-week-old Wistar rat uteri and measured the concentration-dependent substitution of [2,4,5,6-3H]estradiol at the estrogen receptor.

The hormonal activity of the pure substances β-sitosterol, stigmasterol, and their purified chlorine dioxide oxidation products showed estrogenic activity in an estrogen receptor binding assay.[64] In an androgen receptor binding assay, the phytosterols and their oxidation products showed a small but measurable activity.

Four phytostanol mixtures (0, 1, 10 or 100 μmol/L) showed no estrogenic activity in human mammary adenocarcinoma (MCF-7) cells.[55] Estrogenic activity was measured as the ability to induce proliferation of these cells. Proliferation was measured by staining the cells with the protein stain sulforhodamine B and measuring optical density. The MCF-7 cells were cultured for 6 days. 17β-Estradiol was used as a positive control. The percentage of β-sitostanol in the phytostanols, derived from vegetable oil, ranged from 58% - 67%, and campestanol ranged from 29% - 32%. The phytosterol content was < 4%. Precipitation and slight cytotoxicity were observed at the highest test concentration with all mixtures. No cell proliferation was observable in cells treated with phytostanols. Under the conditions of this study, the phytostanol mixtures tested showed no estrogenic activity.

In Vivo

Neither WDPSE nor VODPSE administered in feed (0, 8.3%) for 4 days influenced the uterine weights of female Wistar rats (n = 10; 17-day-old) in a Teicco assay.[55] Diethylstilbestrol (5, 10 or 20 µg/kg) in the diet was used as positive control. Uterine weight was used as an indicator of estrogenic activity. No treatment-related effects on general condition, body weight or food consumption were observed.

β-Sitosterol, stigmasterol, and their oxidation products were inactive in a 28-day mosquito fish masculinization assay at concentrations up to 100 µg/L.[64]

There were no signs of estrogenic activity for phytosterols and phytosterol esters tested in an in vivo immature rat uterotrophic assay (n = 10; up to 500 mg/kg).[63] The phytosterols tested consisted of a mixture of β-sitosterol (47.9%), campesterol (28.8%), and stigmasterol (23.3%) and were sourced from a variety of edible vegetable oil distillates (e.g. sunflower, soya bean and rapeseed oils). The phytosterol esters were prepared by esterifying these phytosterols with fatty acids from sunflower oil.

Absolute and relative uterine weights were unaffected in an immature rat uterotrophic assay of a mixture of phytosterols and phytostanols (0, 500, 1000, 2500 mg/kg) administered twice daily for 4 days when compared with the negative control.[55] The mixture of phytosterols and phytostanols used in this study was derived by solvent extraction (~40–55% β-sitosterol, 16–31% β-sitostanol, 11–15% campesterol and 2–11% campestanol; MPSS-SE) and was assessed using female, Crl:CD (SD)IGS BR VAF/Plus, 19-day-old rats (n = 10). Ethinyl estradiol was used as a positive control. Body weight gains of animals in the 2000 and 5000 mg/kg groups were reduced.

PEG SOY STEROL REPORT

Dose-dependent uterotrophic effects of subcutaneously administered β-sitosterol in ovariectomized rats and its synergism with estradiol could be attributable to intrinsic estrogenic properties; the effects of β-sitosterol could be inhibited by progesterone.[57]

β-Sitosterol was an effective estrogen-like agonist causing vaginal cornification and uterine weight gain in adult, ovariectomized Wistar rats.[65] 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 subcutaneous treatment with β-sitosterol.[57]

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.[57,63,66]

Sulfates of β-sitosterol act as abortifacients in female rats and Dutch-belted rabbits via estrogenic effects. They also exhibit spermicidal effects. β-Sitosterol itself had anti-estrogenic, anti-progestational, gonadotrophic, anti-gonadotrophic, and anti-androgenic effects.[45,67,68]

GENOTOXICITY

In multiple in vitro (up to 5000 µg/plate) and in vivo (up to 2000 mg/kg) assays, phytosterols and phytosterol esters were negative for genotoxicity (Table 8). These tests included reverse mutation, chromosomal aberrations, gene mutation, clastogenicity, sister chromatid exchange (mice), micronucleus induction (rats and mice), and unscheduled DNA synthesis assays (rats).[69-72]

PEG SOY STEROL REPORT

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.[57,73-79]

CARCINOGENICITY

No new published carcinogenicity studies were discovered and no unpublished data were submitted.

PEG SOY STEROL REPORT

Sitosterol inhibited the tumor-promoting activity of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in the skin of female ICR mice after initiation with 7,12-dimethylbenz[a]anthracene (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 ornithine decarboxylase (ODC) activity; ODC induction can represent 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.[45,80]

Male Fischer CD rats coadministered the direct-acting carcinogen N -methylnitrosourea (by cannulation on days 1, 4, 7, 10) and β-sitosterol (95% pure, with 4% campesterol and 1 % stigmasterol; 0.2% in feed for 28 weeks) had significantly fewer colonic tumors (benign or benign and malignant) compared to rats given the carcinogen alone after 28 weeks.[81] 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% (5171) 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).[82,83]

IRRITATION AND SENSITIZATION

Irritation

Dermal – Non-Human

WDPSE (2000 mg/kg) administered to the clipped skin of male albino rabbits (n = 3) for 4 h under semi-occlusion was not irritating.[55] VODPSE caused very slight erythema after 1 h of treatment, which was completely reversed within 24 h after treatment. Skin irritation/corrosion was tested with rabbits in according to OECD Test Guideline 404.

Dermal – Human

Phytosterols (100%; 1 mL; derived from pomegranate) were not irritating to scarified skin in a repeat irritation assay (n = 10).[84] The test site was scratched with a 30-guage needle. The test material was administered to the same scarified location on the forearm, using a chamber, for 24 h for three consecutive days. The site was examined 30 min after removal and before the next treatment.

In-Vitro

In an EpiDerm™ assay, phytosterols (100%) from three sources (derived from pomegranate, soybean, and acai) were not predicted to be dermal irritants.[85-87]

Ocular

There was no irritation potential revealed for WDPSE and VODPSE in a chicken enucleated eye assay.[55]

WDPSE and VODPSE (concentration not provided; assumed 100%) were considered minimally irritating in a Draize assay using albino rabbits (n not provided).[55] The assay was conducted in accordance with OECD Test Guideline 405. WDPSE and VODPSE (concentration not provided; assumed 100%) caused slight and slight or moderate discharge, respectively, which was reversible within 24 h after treatment.

In an EpiOcular™ assay, phytosterols (100%) from three sources (derived from pomegranate, soybean, and acai) were not predicted to be ocular irritants.[85-87]

Sensitization

Non-Human

Neither WDPSE nor VODPSE (concentration not provided) caused signs of skin sensitization after administration to male guinea pigs (n = 10) in a maximization assay conducted in accordance with OECD Test Guideline 406.[55]

Human

There were no signs of irritation or sensitization in a human repeat insult patch test (HRIPT; n = 50) of sterols (100%; 0.2 mL; 0.2 g; derived from pomegranate).[88] The test material was heated to liquefy it, and then it was applied to an occlusive, hypoallergenic patch. The patch was applied to the infrascapular regions of the back for nine treatments. The same concentration and amount of the test substance used in the challenge phase.

None of the subjects with confirmed soy allergies (n = 29) had a positive reaction to a skin prick test of plant stanol ester.[13] An open challenge with plant stanol ester within four weeks of the HRIPT (cumulative dose 5.55g) was negative in 26 of 33 (the original 29 + 4 more) subjects. Positive reactions consisted of itching of the throat in three participants, cutaneous symptoms in three, and loose stools in one subject. The reactions were observed after the final cumulative dose of plant stanol ester; all symptoms resolved without treatment.

A follow-up double-blind placebo controlled food challenge (DBPCFC) study with plant stanol ester performed on 6 of the subjects with positive reactions in the skin prick test had negative results. The DBPCFC with plant stanol ester in the remaining seventh subject (female) was interpreted as negative, although she reported loose stools the morning after the last challenge, which contained plant stanol ester. In view of the cumulative oil intake, a nonimmune-mediated effect may be considered.[13]

Of 22 subjects that had positive reactions to a commercial soy extract in a skin prick test, 16 had a positive reaction to soy isolate and 6 to soy.[19] None had a reaction to phytosterols.

In Vitro

In an immunoblotting assay for soybean proteins using polyclonal, soybean-specific antiserum from rabbits (RBiopharm) and sera from nine soybean-allergic subjects, no soy protein or other protein was detected.[19] Oleosin was added as a control; the oleosin fraction was shown to be a minor IgE-binding constituent of the total soybean protein. The limit of detection was 50 ng of the reference soybean extract and 100 ng of oleosin.

All hydrophilic extracts of vegetable oil deodorized distillate (VOD) samples (n = 9) analyzed by immunoblotting with soy-specific antiserum from rabbits and by IgE-immunoblotting with a pooled human serum detected no soy protein or other protein. There was no IgE binding with the VOD or the phytosterol samples using either the pooled human serum or the serum of one subject who had experienced mild oral allergy syndrome after a DBPCFC with phytosterols. The authors concluded that no IgE-binding proteins were present in the VOD and phytosterol samples at or above 1 and 10 μg/g, respectively.[19]

Refined soybean oils exhibited no detectable IgE binding activity using immunoblotting and enzyme allergosorbent test (EAST) inhibition assays.[89]

CLINICAL USE

Case Studies

A female subject excreted increasing amounts of β-sitosterol, campesterol and stigmasterol through the skin as oral intake of phytosterols increased over sustained periods of time.[90] When phytosterols were removed from the diet, the amount of β-sitosterol in the skin decreased from 6 mg/d to 0.08 mg/d within 83 days and finally became undetectable. Similar results were reported for the other two phytosterols. Twenty days after the administration of 30 g/d phytosterols, β-sitosterol, as well as campesterol and stigmasterol, reappeared in the skin and was excreted at 5 mg/d by 6 weeks.

SUMMARY

A total of 26 phytosterols and steryl alkanoates are described for use in cosmetics. These ingredients are sterols derived from plants, many of which are then esterified with plant-derived fatty acids. These ingredients are reported to function as skin-conditioning agents, hair conditioning agents, viscosity increasing agents, skin protectants, antioxidants, drug astringents, and fragrances.

The Panel concluded that PEG-5, -10, -16, -25, -30, and -40 soy sterols were safe as used in a prior amended safety assessment. Relevant component chemicals that are cosmetic ingredients and have been reviewed by the Panel were all found to be safe as used. Butyric acid, caprylic acid/capric acid, and linoleic acid/linolenic acid have not been reviewed. Octyldecanoic acid is not a cosmetic ingredient.

Phytosterols are from edible plant sources and exposure to phytosterols in food results in a much greater systemic exposure than that resulting from use in cosmetic products containing these ingredients. It was noted in the PEG soy sterol report that phytosterols and phytosterol esters are not significantly absorbed after oral exposure. Therefore, acute and repeated dose oral toxicity potential of these phytosterols were not addressed in this report and the focus is on the potential for reproduction toxicity, genotoxicity, carcinogenicity, irritation, and sensitization.

Protein content of phytosterol blends was not detectable at the detection limits of 10-20 μg/g.

The phytosterols are used in all of the FDA's cosmetic category groups except baby products. They are used at maximum concentrations ranging from 0.000001% - 8%.

Phytosterols are used in food products at up to 5 g/L. The Western diet contains ~160-360 mg/d phytosterols consisting of ~80% β-sitosterol.

Less than 5% of dietary phytosterols, phytostanols, and their esters are absorbed in the gastrointestinal tract of rats and humans.

β-sitosterol (200 μg/mL in ethanol) and β-sitosterol/campesterol (50%/40%; 200 μg/mL in ethanol) were cytotoxic to mouse macrophages in vitro.

The LD_{50} of two mixtures of phytosterol esters was reported to be > 2000 mg/kg.

There were no maternal or teratogenic effects attributed to phytosterol esters administered in the feed of rats in a two-generation study. The NOAEL was ≥8.1%, the highest concentration tested. There were no signs of reproductive toxicity to male and female American minks orally administered β-sitosterol up to 50 mg/kg/d for 10 months.

Subcutaneous injections of β-sitosterol at 5 mg/kg/d for 16 to 48 days reduced sperm concentrations and fertility, and decreased testis and accessory sex tissue weights in a time-dependent manner in male rats.

In multiple in vitro (up to 5000 μg/plate) and in vivo (up to 2000 mg/kg) genotoxicity assays, phytosterols and phytosterol esters were negative. These tests included reverse mutation, chromosomal aberration, gene mutation, clastogenicity, micronucleus induction, and unscheduled DNA synthesis assays.

A phytosterol mixture was not irritating to albino rabbits at 2000 mg/kg.

Two phytosterol mixtures were minimally irritating to albino rabbits.

Phytosterols derived from pomegranate at 100% were not irritating to scarified skin in a human repeat irritation assay.

Two phytosterol mixtures were not sensitizing to guinea pigs. Phytosterols derived from pomegranate were not

sensitizing in and HRIPT at 100%. None of 29 subjects with confirmed soy allergies had a positive reaction to a skin prick test with plant stanol ester. Of 22 subjects that had positive reactions to a commercial soy extract in a skin prick test, none had a reaction to phytosterols.

There were no IgE-binding proteins detected in multiple hydrophilic extracts of vegetable oils samples using immunoblotting or an EAST inhibition assays.

There was little or no estrogenic activity detected in phytosterols using in vitro estrogen binding assays. Two phytosterol ester mixes administered in feed at 8.3% for 4 days did not affect the uterus weights of 17-day-old rats in a Teicco assay. However, in the PEG soy sterol report, β-Sitosterol was an effective estrogen-like agonist causing vaginal cornification and uterine weight gain in adult, ovariectomized Wistar rats.

There were no signs of estrogenic activity in phytosterol mixtures up to 2500 mg/kg in immature rat uterotrophic assays.

DISCUSSION

The Panel noted that phytosterols are naturally-occurring in edible plants and are consumed in a normal diet. Therefore, not considering oral toxicity data and concentrating on reproductive toxicity, genotoxicity, carcinogenicity, irritation, and sensitization was appropriate.

Irritation assays and multiple sensitization assays did not demonstrate any signs of irritation or sensitization up to 100%. There were no positive reactions to phytosterols in a skin prick test using subjects with positive reactions to soy and soy isolate. No maternal or teratogenic effects were discovered from the oral administration of phytosterols. There was no evidence of genotoxicity or carcinogenicity.

After examining the data on estrogenic effects, the Panel had no concern about such effect from dermal exposure to phytosterols.

The Expert Panel expressed concern about pesticide residues and heavy metals that may be present in botanically-derived ingredients. They stressed that the cosmetics industry should continue to use current good manufacturing practices (cGMPs) to limit impurities.

CONCLUSION

The CIR Expert Panel concluded that the following phytosterol ingredients are safe in the present practices of use and concentration described in this safety assessment in cosmetics:

- brassica campestris (rapeseed) sterols
- canola sterols
- C10-40 isoalkyl acid phytosterol esters
- dihydrophytosteryl octyldecanoate
- euterpe oleracea sterols
- glycine soja (soybean) sterols
- persea gratissima (avocado) sterols
- phytosterols
- phytosteryl butyrate
- phytosteryl canolate
- phytosteryl caprylate/caprate
- phytosteryl hydroxystearate
- phytosteryl isostearate
- phytosteryl linoleate
- phytosteryl linoleate/linolenate
- phytosteryl macadamiate
- phytosteryl nonanoate
- phytosteryl oleate
- phytosteryl rice branate
- phytosteryl ricinoleate
- phytosteryl sunflowerseedate
- punica granatum sterols
- beta-sitosterol
- beta-sitosteryl acetate
- soy sterol acetate
- tall oil sterolphytosteryl caprylate/caprate
- phytosteryl hydroxystearate
- phytosteryl isostearate

TABLES

Table 1. Definitions and functions of the phytosterols in this safety assessment.[1] Descriptions provided below in *italics* have been generated by CIR staff.

Ingredient CAS No.	Definition	Function
Brassica campestris (rapeseed) sterols	A mixture of sterols obtained from *Brassica campestris* (rapeseed) Seed Oil. *Rapeseed oil is known to contain brassicasterol, poriferasterol, and campesterol.*[4]	Skin-conditioning agent – emollient
Canola Sterols	A mixture of sterols obtained from the seeds of the canola plant.	Skin-conditioning agent – emollient
C10-40 isoalkyl acid phytosterol esters	A complex mixture of esters of phytosterol and C10-40 isoalkyl acid.	Hair conditioning agent; skin-conditioning agent – emollient; viscosity increasing agent – nonaqueous
Dihydrophytosteryl octyldecanoate	The ester of dihydrophytosterol and branched chain octyldecanoic acid.	Skin conditioning agent – occlusive
Euterpe oleracea sterols	The sterol fraction isolated from the whole plant of *Euterpe oleracea*.	Skin conditioning agent – miscellaneous
Glycine soja (soybean) sterols	A mixture of phytosterols obtained from the soybean, *Glycine soya. Soybean is known to contain stigmasterol.*[4]	Skin-conditioning agent – emollient
Persea gratissima (avocado) sterols	A mixture of sterols obtained from *Persea gratissima* (avocado) oil.	Skin-conditioning agent – emollient
Phytosterols	A mixture of sterols obtained from higher plants.	Skin conditioning agent – miscellaneous
Phytosteryl butyrate	The ester of phytosterols and butyric acid.	Hair conditioning agent; skin-conditioning agent – miscellaneous
Phytosteryl canolate	The ester of phytosterols and the fatty acids derived from canola oil.	Skin protectant; skin-conditioning agent – emollient; viscosity increasing agent – nonaqueous
Phytosteryl caprylate/caprate	The ester of phytosterols with a mixture of caprylic acid and capric acid.	Hair conditioning agent; skin-conditioning agent – occlusive
Phytosteryl hydroxystearate	The ester of phytosterols and hydroxystearic acid.	Skin-conditioning agent – emollient
Phytosteryl isostearate	The ester of phytosterols and isostearic acid.	Hair conditioning agent; skin-conditioning agent – occlusive
Phytosteryl linoleate	The ester of phytosterols with linoleic acid.	Antioxidant
Phytosteryl linoleate/linolenate	The ester of phytosterols with a mixture of linoleic acid and linoleic acid.	Antioxidant
Phytosteryl macadamiate	The ester of phytosterols and the fatty acids derived macadamia seed oil.	Hair conditioning agent; skin-conditioning agent – miscellaneous
Phytosteryl nonanoate	The ester of phytosterols and nonanoic acid.	Hair conditioning agent; skin-conditioning agent – miscellaneous
Phytosteryl oleate	The ester of phytosterols and oleic acid.	Hair conditioning agent; skin-conditioning agent – miscellaneous
Phytosteryl rice branate	The ester of phytosterols and rice bran acid.	Drug astringent – skin protectant drug; hair conditioning agent; humectant; skin protectant; skin-conditioning agent – emollient
Phytosteryl ricinoleate	The ester of phytosterols and ricinoleic acid.	Hair conditioning agent; skin-conditioning agent – miscellaneous
Phytosteryl sunflowerseedate	The ester formed by the reaction of sunflower seed acid with phytosterols.	Skin-conditioning agent – miscellaneous
Punica granatum sterols	A mixture of sterols obtained from *Punica granatum* seed oil.	Hair conditioning agent; ; skin-conditioning agent – emollient; skin-conditioning agent – occlusive
Beta-sitosterol 83-46-5	*A sterol that is found in most plant oils and conforms to the structure in Figure 1.*[4]	Fragrance ingredient; Skin-conditioning agent – miscellaneous
Beta-sitosteryl acetate 915-05-9	The ester of beta-sitosterol and acetic acid *that conforms to the structure in Figure 1.*	Skin-conditioning agent – miscellaneous
Soy sterol acetate	The acetic acid esters of soy sterol.	Skin-conditioning agent – occlusive
Tall oil sterol	The complex mixture of phytosterols (polycyclic polyterpenes, complex monohydric alcohols and their esters) recovered from fractions of tall oil.	Skin-conditioning agent – miscellaneous

Table 2. CIR safety assessments of constituents of phytosterol ingredients.

Constituent	Conclusion	Maximum concentration of use reported	Reference
PEG-5, -10, -16, -25, -30, and -40 soy sterol	Insufficient; Safe as used.	2%	[2,3]
Plant-derived fatty acid oils	Safe as used.	100%	[91]
C10-40 isoalkyl acid	*As C10-40 isoalkyl acid octylodecanol esters, C4-5 isoalkyl cocoate, C32-36 isoalkyl stearate, and ethylhexyl C10-40 isoalkyl acidate.* Safe in the present practices of use and concentration described in this safety assessment when formulated to be non-irritating.	78%	[92]
Octyldecanoic acid	Not a cosmetic ingredient.	-	
Butyric acid	Not reviewed.	-	
Caprylic acid/capric acid	Not reviewed.	-	
Hydroxystearic acid	Safe as used.	10%	[93]
Isostearic acid	Safe as used.	26%	[94,95]
Linoleic acid/linoleic acid	Not reviewed.	-	
Nonanoic acid	*As pelargonic acid.* Safe as used.	74%	[96]
Oleic acid	Safe as used.	43%	[97,98]
Rice bran acid	Safe as used.	100%	[91,99]
Ricinoleic acid	Safe as used.	69%	[100]
Sunflower seed acid	Safe as used.	100%	[91]
Acetic acid	Safe as used.	0.4%	[101]
Tall oil acid	Safe as used.	8%	[102]

Table 3. Chemical characterization of a single sample and multiple samples of plant sterol material (source plant not provided) demonstrating the variation in sterol content.[3,8,9]

	Distribution of phytosterols (%)	
Phytosterol	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
B-Sitosterol	48.7	50.8-51.2
B-Sitostanal	1.8	Not provided
Cholesterol	0.4	0.2-0.3
Other sterols	0.8	1.2-1.7

Table 4. Percent distribution of phytosterols from common vegetable oils.[3,5]

Oil source	Brassicasterol	Campesterol	Stigmasterol	B-Sitosterol	Δ7 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	

Table 5. Chemical and physical properties of representative sterols.

Property	Value	Reference
Vegetable oil sterols		
Physical Form	Crystalline waxy powder or prills	[10]
	Waxy, free-flowing granular powder	[19]
Color	White to off white	[10]
Odor	Vegetable oil-like	[19]
Melting Point °C	138-158	[10]
Water Solubility g/L @	< 0.01	[10]
Other Solubility		[10]
Fat at ambient temperature	2.5%	
Acetone	Soluble	
Ethyl acetate	Soluble	
Isopropanol	Soluble	
Tall oil sterols/stanols		
Physical Form	Crystalline waxy powder or prills	[10]
Color	White to off white	[10]
Melting Point °C	138-158	[10]
Water Solubility g/L @ °C & pH	< 0.01	[10]
Other Solubility		[10]
Fat at ambient temperature	2.5%	
Acetone	Soluble	
Ethyl acetate	Soluble	
Isopropanol	Soluble	

Table 6. Comparison of phytosterol content of tall oil extracted by simpler saponification process and a more complicated, multi-step processes.[4]

Phytosterol	Saponification process (%)	Multi-step process (%)
Total phytosterols	98.1	99.7
Major phytosterols	88.7	92.7
β-Sitosterol	49.1	59.8
β-Sitostanol	19.9	23.2
Campesterol	15.0	6.5
Stigmasterol	< 1%	< 1%
Other phytosterols	9.3 (including stigmasterol)	7.0 (including stigmasterol)

Table 7. Allergens not detected in phytosterols derived from acai, soybean, and pomegranate.[20-22]

Alpha-isomethyl ionone	Amyl cinnamal	Anise alcohol
Benzyl alcohol	Benzyl benzoate	Benzyl cinnamate
Benzyl salicylate	Butylphenyl methylpropional	Cinnamal
Cinnamyl alcohol	Citral	Citronellol
Coumarin	Eugenol	Fanesol
Geraniol	Hexyl Cinnamal	Hydroxycetronellal
Hydroxymethylpentyl 3-cyclohexene carboxaldehyde	Isoeugenol	Limonene
Linalool	Methyl 2 octynoate	Evernia prunastri
Evernia furfuracea	Amylcinnamyl alcohol	

Table 8. Frequency of use according to duration and exposure of phytosterols.[24,25]

Use type	Uses	Maximum Concentration (%)	Uses	Maximum Concentration (%)	Uses	Maximum Concentration (%)	Uses	Maximum Concentration (%)
	Brassica campestris (rapeseed) sterols		Euterpe oleracea sterols		Glycine soja (soybean) sterols		Persea gratissima (avocado) sterols	
Total/range	**57**	**0.0008-7**	**2**	**NR**	**240**	**0.000001-4.1**	**NR**	**0.1-1**
Duration of use								
Leave-on	50	0.0008-7	2	NR	194	0.000001-1	NR	0.1-1
Rinse-off	7	0.0055-0.13		NR	45	0.000001-4.1	NR	NR
Diluted for (bath) use	NR	NR	NR	NR	1	NR	NR	NR
Exposure type								
Eye area	3	0.005	NR	NR	17	0.001-1	NR	1
Incidental ingestion	2	0.0008-7	1	NR	3	0.1-1	NR	0.65
Incidental Inhalation-sprays	2	NR	NR	NR	6	0.00000-0.001	NR	NR
Incidental inhalation-powders	NR	NR	NR	NR	1	0.001-0.1	NR	NR
Dermal contact	54	0.0055-0.5	1	NR	193	0.001-4.1	NR	0.1-1
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair-noncoloring	NR	0.13	NR	NR	43	0.000001-0.018	NR	NR
Hair-coloring	NR	NR	NR	NR	NR		NR	NR
Nail	1	NR	NR	NR	NR	1	NR	NR
Mucous Membrane	4	0.0008-7	1	NR	10	0.01-1	NR	0.65
Baby	NR	NR	NR	NR	NR	NR	NR	NR

	Phytosterols		Phytosteryl canolate		Phytosteryl isostearate		Phytosteryl [phytosterol] macadamiate	
Total/range	**403**	**0.0001-5**	**NR**	**0.06**	**16**	**0.003-3**	**183**	**0.001-8**
Duration of use								
Leave-on	177	0.0001-5	NR	0.06	15	0.003-3	181	0.001-8
Rinse-off	215	0.00018-0.5	NR	NR	1	0.5	2	0.01-1
Diluted for (bath) use	11	NR	NR	NR	NR	NR	NR	NR
Exposure type								
Eye area	5	0.00018-2	NR	0.06	4	0.003-0.5	2	0.01-3
Incidental ingestion	63	0.01-5	NR	NR	8	2.8-3	100	4.1-7
Incidental Inhalation-sprays	2	0.0001	NR	NR	NR	NR	NR	NR
Incidental inhalation-powders	1	0.05	NR	NR	NR	NR	NR	0.001
Dermal contact	338	0.0001-3.2	NR	0.06	8	0.003-1	82	0.001-8
Deodorant (underarm)	NR	0.06	NR	NR	NR	NR	NR	NR
Hair-noncoloring	2	0.5-2.4	NR	NR	NR	0.1	1	0.01-1
Hair-coloring	NR	NR	NR	NR	NR	NR	NR	
Nail	NR	NR	NR	NR	NR	NR	NR	0.01
Mucous Membrane	280	0.0002-5	NR	NR	8	2.8-3	100	4.1-7
Baby	NR	NR	NR	NR	NR	NR	NR	NR

Table 8. Frequency of use according to duration and exposure of phytosterols.[24,25]

Use type	Uses	Maximum Concentration (%)	Uses	Maximum Concentration (%)	Uses	Maximum Concentration (%)	Uses	Maximum Concentration (%)
	Phytosteryl oleate		**Phytosteryl rice branate**		**Punica granatum sterols**		**Beta-sitosterol**	
Total/range	**26**	**1.5-3**	**2**	**0.5-1**	**31**	**0.001-5**	**48**	**0.00007-0.06**
Duration of use								
Leave-on	20	1.5-3	NR	0.5-1	29	0.1-5	46	0.00007-0.06
Rinse-off	6	NR	NR	NR	2	NR	2	NR
Diluted for (bath) use	NR	NR	NR	NR	NR	0.001	NR	NR
Exposure type								
Eye area	1	NR	1	1	3	NR	3	
Incidental ingestion	NR	1.5	NR	NR	14	0.1-5	1	0.00007-0.0008
Incidental Inhalation-sprays	NR	NR	NR	NR	NR	NR	4	NR
Incidental inhalation-powders	NR	NR	NR	NR	NR	NR	NR	0.0021
Dermal contact	26	3	2	0.5-1	15	0.001-0.5	47	0.0004-0.06
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair-noncoloring	NR	NR	NR	NR	2	NR	NR	NR
Hair-coloring	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	1.5	NR	NR	15	0.001-5	1	0.00007-0.0008
Baby	NR	NR	NR	NR	NR	NR	NR	NR

	Soy sterol acetate		**Tall oil sterol**	
Total/range	**1**	NR	**7**	**0.0006-0.0046**
Duration of use				
Leave-on	1	NR	7	0.0045-0.0046
Rinse-off	NR	NR	NR	0.0006
Diluted for (bath) use	NR	NR	NR	NR
Exposure type				
Eye area	NR	NR	NR	NR
Incidental ingestion	NR	NR	NR	NR
Incidental Inhalation-sprays	NR	NR	NR	NR
Incidental inhalation-powders	NR	NR	NR	NR
Dermal contact	1	NR	7	0.0006-0.0046
Deodorant (underarm)	NR	NR	NR	NR
Hair-noncoloring	NR	NR	NR	NR
Hair-coloring	NR	NR	NR	NR
Nail	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR
Baby	NR	NR	NR	NR

NR = Not Reported; NS = Not Surveyed; Totals = Rinse-off + Leave-on Product Uses.
Note: Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure type uses may not equal the sum total uses.

Table 9. Genotoxicity assays of phytosterols.

Assay	Test material(s) (concentration)	Results	Reference
In vitro			
Reverse mutation *Salmonella typhimurium* (strains TA98, TA100, TA102)	7-ketositosterol (up to 5% in acetone/tween80, 3:1 v/v), 7β-OH-sitosterol (up to 5%), 7α-OH-sitosterol (up to 1%), 6α-OH-3-keto-/6β-OH-3-ketositosterol (ratio 4:3; up to 2.5%) and a mixture (up to 10%)	Negative with and without metabolic activation	[69]
Reverse mutation S. *typhimurium* (TA98, TA100, TA1535 and TA1537)	Phytosterol mixture[a] (5–5000 µg/plate)	Negative with and without metabolic activation	[72]

Table 9. Genotoxicity assays of phytosterols.

Assay	Test material(s) (concentration)	Results	Reference
Reverse mutation; *S. typhimurium* (TA98, TA100, TA1535 and TA1537; *Escherichia coli* WP2 uvrA (pKM101)	Phytosterol esters[a] (50-5000 µg/plate)	Negative with and without metabolic activation	72
Reverse mutation *S. typhimurium* (TA98, TA100, TA102, TA1535 and TA1537)	Phytosterol oxide concentrate from vegetable oil distillates (1.6-5000 µg/plate)	Negative with and without metabolic activation	70
Reverse mutation *S. typhimurium* (TA98, TA100, TA1535 and TA1537); *E. coli* WP2 uvrA	MPSS-SE[c] (104-1667 µg/plate)	Negative with and without metabolic activation	55
Reverse mutation *S. typhimurium* (TA98, TA100, TA1535, and TA1537); *E. coli* WP2 uvrA	MPSS-VD[d] (16-1000 µg/plate)	Negative with and without metabolic activation	55
Reverse mutation S. *typhimurium* (TA98, TA100, TA1535 and TA1537)	WDPSE[e] (62-5000 µg/plate)	Negative with and without metabolic activation	55
Reverse mutation S. *typhimurium* (TA98, TA100, TA1535 and TA1537)	VODPSE[f] (62-5000 µg/plate)	Negative with and without metabolic activation	55
Reverse mutations histidine-dependent *S. typhimurium* (TA98, TA100, TA1535, TA1537); tryptophan-dependent *E. coli* (WP2uvrA)	Pomeganate sterols (50 mg/mL; 0.1 mL)	Negative with and without metabolic activation	103
Chromosomal aberration; Human peripheral blood lymphocytes	Phytosterol mixture[a] (40-160 µg/mL)	Negative with and without metabolic activation	72
Chromosomal aberration; Human peripheral blood lymphocytes	Phytosterol esters[a] (25-200 µg/mL)	Negative with and without metabolic activation	72
Chromosomal aberration; Human peripheral blood lymphocytes	Phytosterol oxide concentrate[g] (131.1-500 µg/mL)	Negative with and without metabolic activation	72
Chromosomal aberration; Human peripheral blood lymphocytes	MPSS-SE (100-1200 µg/mL)	Negative with and without metabolic activation	55
Chromosomal aberration; Human peripheral blood lymphocytes	MPSS-VD (31.3-1000 µg/mL)	Negative with and without metabolic activation	55
Chromosomal aberration; Chinese hamster ovary cells	WDPSE (up to 500 µg/ml)	Negative with and without metabolic activation	55
Chromosomal aberration; Chinese hamster ovary cells	VODPSE (up to 2000 µg/ml)	Negative with and without metabolic activation	55
Gene mutation; Mouse lymphoma L5178Y cells, *Tk*+/- locus	Phytosterol esters[a] (5-80 µg/mL)	Negative with and without metabolic activation	70
Gene mutation; Mouse lymphoma L5178Y cells, *Tk*+/- locus	MPSS-SE (5-167 µg/mL)	Negative with and without metabolic activation	55
Gene mutation; Mouse lymphoma L5178Y cells, *Tk*+/- locus	WDPSE (20-500 µg/ml)	Negative with and without metabolic activation	55
Gene mutation; Mouse lymphoma L5178Y cells, *Tk*+/- locus	VODPSE (125-3000 µg/ml)	Negative with and without metabolic activation	55
Clastogenicity (micronucleus induction); Human peripheral blood lymphocytes	Phytosterol oxide concentrate[g] (up to 625 µg/mL)	Negative with and without metabolic activation	70
In vivo			
Micronucleus induction; male rats, bone marrow	Phytosterol esters[b] (500-2000 mg/kg/d) for 2 days	Negative	72
Micronucleus induction; male and female rats, bone marrow	MPSS-SE (50, 500, 2000 mg/kg)	Negative	55
Unscheduled DNA synthesis; male rats, liver	Phytosterol esters[b] (800, 2000 mg/kg)	Negative	72
Micronucleus induction; male mice, blood	Triols (up to 9.4 mg/kg) and epoxides of a mixture of β-sitostrol and campesterol (67 mg/kg)	Negative	104
Sister chromatid exchange; male NIH mice (8 weeks old)	β-sitostrol (200, 400, 600, 1000 mg/kg)	Negative	71
Cellular proliferation kinetics; male NIH mice (8 weeks old)	β-sitostrol (200, 400, 600, 1000 mg/kg)	Negative	71
Mitotic index; male NIH mice (8 weeks old)	β-sitostrol (200, 400, 600, 1000 mg/kg)	Negative	71
Micronucleated polychromatic erythrocytes; male NIH mice (8 weeks old)	β-sitostrol (200, 400, 600, 1000 mg/kg)	Negative	71

[a] Phytosterol composition: campesterol (26.7%), stigmasterol (17.7%), β-sitosterol (51%).
[b] Phytosterol composition: campesterol (28.1%), stigmasterol (18.7%), β-sitosterol (45.5%)
[c] MPSS-SE = Mixture of phytosterols and phytostanols derived from solvent extraction, which consisted of ~40– 55% β-sitosterol, ~16–31% β-sitostanol, ~11–15% campesterol, and ~2–11% campestanol.
[d] MPSS-VD = Mixture derived from vacuum distillation which consisted of ~63.5% β-sitosterol, ~21.7% β-sitostanol,~ 6.5% campesterol and ~2.8% campestanol.
[e] WDPSE = A wood-derived stanol mixture which consisted of ~94% β-sitostanol and ~6% campestanol.
[f] VODPSE = A vegetable oil–derived mixture of phytostanol esters which consisted of ~68% β-sitostanol and ~32% campestanol.
[g] Phytosterol oxide concentrate = ~ 30% phytosterol oxides.

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