Safety Assessment of Phytosteryl Glutamates as Used in Cosmetics

Status: Final Report
Release Date: July 10, 2023
Panel Meeting Date: June 12-13, 2023

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ABBREVIATIONS

ATP	adenosine triphosphate
CFR	Code of Federal Regulations
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
CPSC	Consumer Product Safety Commission
DPRA	direct peptide reactivity assay
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
HRIPT	human repeated insult patch test
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide
NOEL	no observed effect level
OECD	Organisation for Economic Co-operation and Development
NR	not reported
PEG	polyethylene glycol
SIOPT	single insult occlusive patch test
SLS	sodium lauryl sulfate
t_{50}	duration of exposure resulting in a 50% decrease in MTT conversion
TG	test guideline
US	United States
VCRP	Voluntary Cosmetic Registration Program
w/w	weight for weight
WHO	World Health Organization
wINCI; *Dictionary*	web-based *International Cosmetic Ingredient Dictionary and Handbook*

ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of 3 phytosteryl glutamates as used in cosmetic formulations. All of these ingredients are reported to function as skin conditioning agents in cosmetics. The Panel reviewed the relevant data to determine the safety of these ingredients. Industry should continue to minimize impurities that could be present in cosmetic formulations, such as heavy metals, according to limits set by the Food and Drug Administration (FDA) and the Environmental Protection Agency (EPA). The Panel concluded that the 3 phytosteryl glutamates are safe in the present practices of use and concentration described in this safety assessment.

INTRODUCTION

The safety of the following 3 phytosteryl glutamates as used in cosmetics is reviewed in this safety assessment.

Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate
Phytosteryl/Behenyl/Octyldodecyl/Isostearyl Lauroyl Glutamate
Phytosteryl/Octyldodecyl Lauroyl Glutamate

According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*), all 3 phytosteryl glutamates are reported to function in cosmetics as skin conditioning agents.[1] Phytosteryl/Behenyl/Octyldodecyl/ Isostearyl Lauroyl Glutamate is also reported to function as a hair conditioning agent (Table 1). These ingredients are reviewed together herein as each is a mixture of esters comprising phytosterols, octyldodecanol (and other respective fatty alcohols), and lauroyl glutamic acid.

The Expert Panel for Cosmetic Ingredient Safety (Panel) previously reviewed the safety of the components of these mixed esters. Specifically, the Panel issued a final report on phytosterols, which included phytosteryl isostearate and other phytosteryl alkanoates.[2] The phytosterols ingredient group was considered safe in the present practices of use and concentration (as described in that safety assessment). Safety assessments of behenyl, and isostearyl alcohol found these cosmetic ingredients were safe as used.[3] In subsequent rereviews of these ingredients, the Panel reaffirmed the original conclusions.[4,5] Lauroyl glutamic acid was reviewed as part of the safety assessment of amino acid alkyl amides that was published by the Panel in 2017; the Panel concluded that the amino acid alkyl amides are safe in the present practices of use and concentration in cosmetics when formulated to be non-irritating.[6] At the time of the assessment lauroyl glutamic acid was not in current use, but the Panel stated the conclusion would apply to its safety if used in product categories and at concentrations comparable to others in the group (as described in the safety assessment). The full reports on these ingredients can be accessed on the Cosmetic Ingredient Review (CIR) website (https://www.cir-safety.org/ingredients).

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an extensive search of the world's literature; this search was last performed March 2023. A listing of the search engines and websites that are used, and the sources that are typically explored, as well as the endpoints that the Panel typically evaluates, is provided on the CIR website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data may be provided by the cosmetics industry, as well as by other interested parties.

CHEMISTRY

Definition and Structure

The definitions of the phytosteryl glutamates included in this safety assessment are presented in Table 1.[1] As noted, each of these ingredients comprises 2 core chemical structural residues, phytosterols and lauroyl glutamate. These ingredients also comprise certain fatty alkyl chains. The "/" in the names of these ingredients signifies mixtures. For example, Phytosteryl/Octyldodecyl Lauroyl Glutamate is a mixture of phytosteryl lauroyl glutamate and octyldodecyl lauroyl glutamate. Additionally, according to technical names in the *Dictionary* monograph, the phytosterol components comprise, *inter alia*, campesterol, stigmasterol, and β-sitosterol.[7] These are illustrated in Figure 1, as is an example of connectivity with lauroyl glutamate.

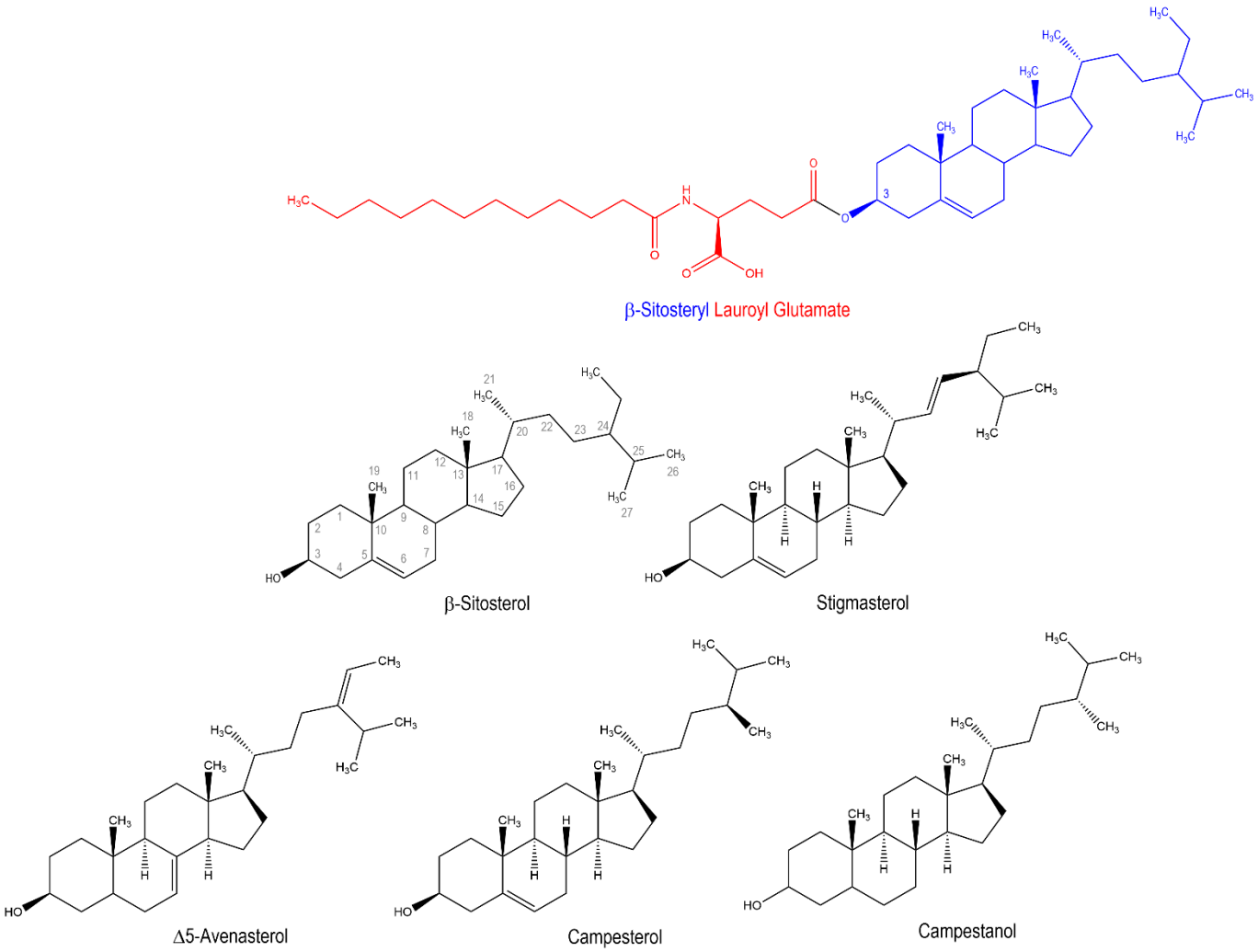

Figure 1. Phytosterols and phytosteryl connectivity

All such connectivities are the result of esterification via the 3-position alcohol functional group of one or more phytosterols. The connectivity of various fatty alkyl chains with lauroyl glutamate is similarly the result of esterification (e.g., octyldodecyl lauroyl glutamate (Figure 2)).

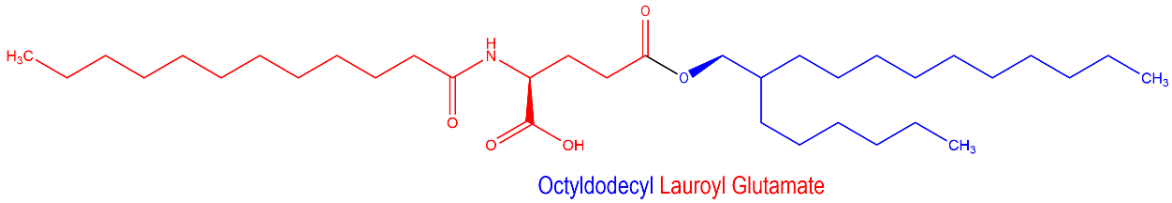

Figure 2. Octyldodecyl Lauroyl Glutamate

Accordingly, Phytosteryl/Octyldodecyl Lauroyl Glutamate is a mixture potentially comprising all of the above instances of esterified lauroyl glutamate. Likewise, Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate and Phytosteryl/Behenyl/ Octyldodecyl/Isostearyl Lauroyl Glutamate comprise similar mixtures.

Chemical Properties

Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate

Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate is a white solid.[8] Results of gel permeation chromatography of Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate in tetrahydrofuran are found in Table 2. The standard was polystyrene.

Method of Manufacture

According to industry, the method of manufacture of each of the phytosteryl glutamates is similar, and only differs by which alcohols are added during esterification.[9] Manufacturing begins with esterification of lauroyl glutamic acid with phytosterol, behenyl alcohol, octyldodecanol, and/or isostearyl alcohol (as appropriate per ingredient) by an acid catalyst. The resulting mixture of esters is purified with an alkaline aqueous solution to remove lauroyl glutamic acid, the acid catalyst, and salts.

Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate

Another method of manufacture of Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate involves the synthesis, cooling, washing, and drying of the ingredient.[8] This is followed by quality control and packing.

Impurities

The levels of heavy metals (as lead (Pb)) in the final product for all three phytosteryl glutamates, when manufactured via the method described above, are less than 20 ppm, and levels of arsenic (as As_2O_3) are less than 2 ppm.[9] The possibility of pesticide contamination is low.

Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate

In an analysis of Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate, where the detection limit was 1 µg/g for arsenic and 2 µg/g for lead, neither arsenic nor lead was detected.[8] The loss on drying of Phytosteryl/Behenyl/ Octyldodecyl Lauroyl Glutamate (105°C, for 1 h) was 0.1%.

USE

Cosmetic

The safety of the cosmetic ingredients addressed in this assessment is evaluated based on data received from the US FDA and the cosmetics industry on the expected use of these ingredients in cosmetics, and does not cover their use in airbrush delivery systems. Data are submitted by the cosmetic industry via the FDA's Voluntary Cosmetic Registration Program (VCRP) database (frequency of use) and in response to a survey conducted by the Personal Care Products Council (Council) (maximum use concentrations). The data are provided by cosmetic product categories, based on 21CFR Part 720. For most cosmetic product categories, 21CFR Part 720 does not indicate type of application and, therefore, airbrush application is not considered. Airbrush delivery systems are within the purview of the US Consumer Product Safety Commission (CPSC), while ingredients, as used in airbrush delivery systems, are within the jurisdiction of the FDA. Airbrush delivery system use for cosmetic application has not been evaluated by the CPSC, nor has the use of cosmetic ingredients in airbrush technology been evaluated by the FDA. Moreover, no consumer habits and practices data or particle size data are publicly available to evaluate the exposure associated with this use type, thereby preempting the ability to evaluate risk or safety.

According to 2023 FDA VCRP data, Phytosteryl/Octyldodecyl Lauroyl Glutamate has the greatest frequency of use; it is reported to be used in 327 cosmetic products, 312 of which are leave-on products and over a third of which are in lipstick formulations (Table 3).[10] The results of the concentration of use survey conducted by the Council in 2021 indicate that Phytosteryl/Behenyl/Octyldodecyl/Isostearyl Lauroyl Glutamate has the highest concentration of use; it is used at maximum use concentrations up to 25.6% in leave-on products (rouges).[11] The maximum concentration of use reported for Phytosteryl/ Octyldodecyl Lauroyl Glutamate is very similar; it is reported to be used at up to 25% in rouges and in lipsticks.

Cosmetic products containing phytosteryl glutamates may incidentally come in contact with the eyes (e.g., Phytosteryl/ Octyldodecyl Lauroyl Glutamate at concentrations up to 12% in eye shadows), and all 3 of these ingredients are reported to be used in formulations that could be incidentally ingested and that come in contact with mucous membranes (e.g., Phytosteryl/ Octyldodecyl Lauroyl Glutamate at concentrations up to 25% in lipstick). Use in baby products is also reported (e.g., Phytosteryl/Octyldodecyl Lauroyl Glutamate is used at up to 0.3% in baby lotions, oils, and creams).

Some of these ingredients are used in cosmetic products that could possibly be inhaled; for example, Phytosteryl/Octyl-dodecyl Lauroyl Glutamate is reported to be used in aerosol deodorant at up to 0.1% and in face powders at concentrations up to 5%. In practice, as stated in the Panel's respiratory exposure resource document (https://www.cir-safety.org/cir-findings), most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and tracheobronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount. There is some evidence indicating that deodorant spray products can release substantially larger fractions of particulates having aerodynamic equivalent diameters in the range considered to be respirable. However, the information is not sufficient to determine whether significantly greater lung exposures result from the use of deodorant sprays, compared to other cosmetic sprays. Conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace.

Although products containing some of these ingredients may be marketed for use with airbrush delivery systems, this information is not available from the VCRP or the Council survey. Without information regarding the frequency and

concentrations of use of these ingredients (and without consumer habits and practices data or particle size data related to this use technology), the data are insufficient to evaluate the exposure resulting from cosmetics applied via airbrush delivery systems.

The phytosteryl glutamates reviewed in this safety assessment are not restricted from use in any way under the rules governing cosmetic products in the European Union.[12]

TOXICOKINETIC STUDIES

Data on toxicokinetic effects of phytosteryl glutamate ingredients reviewed in this safety assessment were neither found in the published literature, nor were these data submitted.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Details regarding the acute dermal and oral toxicity studies summarized below can be found in Table 4.

An acute dermal LD_{50} of > 2000 mg/kg was established for rats given Phytosteryl Octyldodecyl Lauroyl Glutamate.[13] In an acute oral toxicity assay using Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate performed in ICR mice, the oral LD_{50} was reported to be > 2000 mg/kg. Furthermore, an LD_{50} of > 2000 mg/kg was established in an acute oral toxicity study evaluating Wistar rats dosed with 2000 mg/kg of Phytosteryl/Behenyl/Octyldodecyl/Isostearyl Lauroyl Glutamate. In addition, in rats, the acute oral toxicity of Phytosteryl/Octyldodecyl Lauroyl Glutamate was > 2000 mg/kg.

Short-Term Toxicity Studies

Oral

Phytosteryl/Octyldodecyl Lauroyl Glutamate

In a short-term oral toxicity study, a daily dose of Phytosteryl/Octyldodecyl Lauroyl Glutamate was administered by gavage to SPF-bred Wistar rats of both sexes at dose levels of 50, 200, or 1000 mg/kg for 28 d.[14] During the experiment, clinical signs, outside cage observation, food consumption, and body weights were recorded. Functional observational battery, locomotor activity, and grip strength were performed during week 4. After the dosing period, blood samples were drawn for hematology and blood chemistry profile. Histological examinations were performed on organs and tissues. No test substance-related clinical signs were noted, along with no changes in functional observational battery, grip strength, locomotor activity, food consumption, and body weight. Changes in hematology or clinical chemistry parameters were also not reported. There were no reported experimental effects on organ weights; macroscopic and microscopic examination found no changes in experimental animals.

Subchronic and Chronic Toxicity Studies

Data on the subchronic and chronic toxicity of the phytosteryl glutamates reviewed in this safety assessment were neither found in the published literature, nor were these data submitted.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Data on the developmental and reproductive toxicity of phytosteryl glutamates reviewed in this safety assessment were neither found in the published literature, nor were these data submitted.

GENOTOXICITY STUDIES

Details regarding the in vitro genotoxicity studies that are summarized below can be found in Table 5.

No mutagenicity was observed in reverse mutation assays performed on the 3 phytosteryl glutamates (Phytosteryl/ Behenyl/Octyldodecyl, maximum dose 1250 µg/plate; Phytosteryl/Behenyl/Octyldodecyl/Isostearyl Lauroyl Glutamate, maximum dose 5000 µg/plate; Phytosteryl/Octyldodecyl Lauroyl Glutamate, maximum dose 5000 µg/plate) using *Salmonella typhimurium* and *Escherichia coli* strain WP2 uvrA with and without metabolic activation.[13,15] An in vitro chromosome aberration assay conducted on Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate to induce structural chromosomal aberrations in a Chinese hamster lung cell line (CHL/IU) at concentrations between 0.625 - 5.0 µg/ml was negative.[13] Similarly, in two in vitro assays to assess the potential of Phytosteryl/Octyldodecyl Lauroyl Glutamate to induce chromosome aberrations in Chinese hamsters V79 cells (max dose 2500 µg/ml), the test substance was considered to be non-clastogenic.[13,16] A negative result was also observed in an in vitro gene mutation test in mouse lymphoma cells on Phytosteryl/Octyldodecyl Lauroyl Glutamate at concentrations up to 5000 ug/ml when exposed for 4 h.[13]

OTHER RELEVANT STUDIES

Plant Sterols and Sitosterolemia

Phytosteryl glutamates are comprised of 2 core chemical structural residues, phytosterols and lauroyl glutamate.[17] In the intestine, the transporter ABCG5GA is an ATP-binding cassette (ABC) transporter that transports plant sterols from the enterocytes back into the gut. Mutations in the ABC transporters ABCG5 and ABCG8 have been shown to lead to the

accumulation of plant sterols causing the disorder sitosterolemia. Individuals with sitosterolemia exhibit hyperabsorption of β-sitosterol, as well as other sterols, and have markedly reduced secretion of sterols into the bile.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Details regarding the dermal irritation and sensitization studies that are summarized below can be found in Table 6.

Phytosteryl/Behenyl/Octyldodecyl/Isostearyl Lauroyl Glutamate (concentration not stated) was predicted to be non-corrosive in an in vitro human skin model test.[13] In an EpiDerm™ skin irritation assay, the irritation potential of the same ingredient (concentration not stated) was not classified as an irritant. In an in vitro cell viability assay using EpiSkin™ reconstituted human epidermis, a product containing 1% Phytosteryl/Octyldodecyl Lauroyl Glutamate was predicted to be non-irritating.[18] The skin irritation potential of all 3 phytosteryl glutamates (each tested at 100%) was evaluated in studies using New Zealand white rabbits (groups ranging from 3 - 6 animals/study) conducted with a semi-occlusive or occlusive patch; all 3 ingredients were found to be non-irritating.[13] A 14-d open-application skin irritation study yielded no skin reactions on 10 female guinea pigs for Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate and Phytosteryl/Octyldodecyl Lauroyl Glutamate at maximum concentrations of 100%.

In a 24-h occlusive patch test, a Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate cream (applied neat; concentration of Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate not specified) tested on 31 human subjects was deemed to be non-irritating.[8] Two separate 24-h patch tests of Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate and Phytosteryl/ Octyldodecyl Lauroyl Glutamate (both at 100%) were negative for irritation in 45 human subjects.[13] In a human cumulative irritation patch test with 25 subjects that took place over 7 d, a face cream containing 1% Phytosteryl/ Behenyl/Octyldodecyl Lauroyl Glutamate was determined to be non-irritating.[19] A 7-d semi-occlusive cumulative irritation patch test with a formulation containing 1.5% Phytosteryl/Octyldodecyl Lauroyl Glutamate was performed with 38 subjects; no irritation was observed.[20]

In a direct peptide reactivity assay (DPRA), Phytosteryl/Octyldodecyl Lauroyl Glutamate, prepared as a 100 mM stock solution and tested for cysteine and lysine depletion, was predicted to have minimal reactivity, with a mean depletion of 1.8% for cysteine and 0.1% for lysine.[21] Three separate guinea pig maximization tests (15 female guinea pigs/test group) were negative when performed on Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate (25% intradermal induction, 100% epidermal induction, 100% challenge); Phytosteryl/Behenyl/Octyldodecyl/Isostearyl Lauroyl Glutamate (10% intradermal induction, 100% epidermal induction, 50 and 100% challenge); Phytosteryl/Octyldodecyl Lauroyl Glutamate (5% intradermal induction, 100% epidermal induction, 10% challenge).[13] In a human repeated insult patch test (HRIPT), a face cream containing 5% Phytosteryl/Behenyl/ Octyldodecyl Lauroyl Glutamate (102 subjects, tested neat, occlusive patch) was not an irritant or a sensitizer.[22] In another HRIPT, a mixture containing 5.999% Phytosteryl/Octyldodecyl Lauroyl Glutamate (219 subjects; tested neat, occlusive patch) was not an irritant or a sensitizer.[23]

OCULAR IRRITATION STUDIES

Details regarding the ocular irritation studies summarized below can be found in Table 7.

A tissue equivalent assay, measuring the conversion of 3-[4,5,-dimethylthiazol-2-y1]-2,5-diphenyltetrazolium bromide (MTT) by EpiOcular™ cultures was performed to evaluate the ocular irritancy potential of a face cream containing 1% Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate; the t_{50} (duration of exposure resulting in a 50% decrease in MTT conversion) was > 24 h. [24] In another in vitro assay, Phytosteryl/Behenyl/Octyldodecyl/Isostearyl Lauroyl Glutamate (concentration not stated) was found to be a non/minimal irritant.[13] All three phytosteryl glutamates (maximum concentration 100%), when tested as a single instillation into the eyes of New Zealand White rabbits (groups ranging from 3 - 6 animals), were negative for eye irritation.

CLINICAL STUDIES

Phytosteryl/Octyldodecyl Lauroyl Glutamate

In a human in-use test, a product containing 0.5% Phytosteryl/Octyldodecyl Lauroyl Glutamate was applied to the eye area and lashes by 30 female subjects to assess skin and eye acceptability.[25] A pea-sized amount was applied to the eye area each morning and evening, and the product was swiped along the lash line each evening. On day 1, before the first application, and on day 29, a clinical examination of the skin was performed by a dermatologist and of the eyes was performed by an ophthalmologist. No adverse clinical signs were observed by the dermatologist or the ophthalmologist after 28 d of use, and no skin or eye discomfort was reported by the subjects.

SUMMARY

The safety of 3 phytosteryl glutamates as used in cosmetics is reviewed in this safety assessment. According to the *Dictionary*, Phytosteryl/Octyldodecyl Lauroyl Glutamate and Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate are reported to function in cosmetics as skin conditioning agents and Phytosteryl/Behenyl/Octyldodecyl/Isostearyl Lauroyl Glutamate is reported to function as a hair conditioning agent and skin conditioning agent.

According to 2023 FDA VCRP data, Phytosteryl/Octyldodecyl Lauroyl Glutamate has the greatest frequency of use; it is reported to be used in 327 cosmetic products, (312 leave-on products and 15 rinse-off products). The results of a concentration of use survey conducted by the Council in 2021 indicate Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate has the highest concentration of use; it is used at maximum use concentrations up to 25.6% in leave-on products (rouges). The maximum concentration of use reported for Phytosteryl/Octyldodecyl Lauroyl Glutamate is very similar; it is reported to be used at up to 25% in rouges and in lipsticks.

In an acute dermal toxicity study, Phytosteryl Octyldodecyl Lauroyl Glutamate had an LD_{50} of >2000 mg/kg in rats. In acute oral toxicity studies, Phytosteryl/Behenyl/Octyldodecyl/Lauroyl Glutamate had an LD_{50} of 2000 mg/kg in mice while Phytosteryl/Behenyl/Isostearyl/Lauroyl Glutamate and Phytosteryl/Octyldodecyl/Lauroyl Glutamate had an LD_{50} of > 2000 mg/kg in rats.

In two short-term oral toxicity studies, Phytosteryl/Octyldodecyl Lauroyl Glutamate was administered by gavage to SPF-bred Wistar rats of both sexes at dose levels of 50 - 1000 mg/kg for 28 d. In one study, no experimental substance-related clinical signs were noted, along with no changes in functional observational battery, grip strength, locomotor activity, food consumption, and body weight were noted. Changes in hematology or clinical chemistry parameters, organ weights, or macroscopic and microscopic findings were also not observed. The NOEL was observed to be 1000 mg/kg.

No mutagenicity was observed in reverse mutation assays performed on the 3 phytosteryl glutamates (Phytosteryl/Behenyl/Octyldodecyl, maximum dose 1250 µg/plate; Phytosteryl/Behenyl/Octyldodecyl/Isostearyl Lauroyl Glutamate, maximum dose 5000 µg/plate; Phytosteryl/Octyldodecyl Lauroyl Glutamate, maximum dose 5000 µg/plate) using *S. typhimurium* and *E. coli* strain WP2 uvrA with and without metabolic activation. No chromosomal aberrations were noted in Chinese hamster lung cells in an assay conducted on Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate (0.625 - 5.0 µg/ml). Similarly, no chromosomal aberrations were noted in Chinese hamster V79 cells (maximum dose 2500 µg/ml) in two in vitro assays. A negative result was also observed in an in vitro gene mutation test in mouse lymphoma L5178Y/TK cells on Phytosteryl/Octyldodecyl Lauroyl Glutamate at concentrations up to 5000 µg/ml when exposed for 4 h.

Phytosteryl glutamates are comprised of 2 core chemical structural residues, phytosterols and lauroyl glutamate. Mutations in the ABC transporters ABCG5 and ABCG8 have been shown to lead to the accumulation of plant sterols causing the disorder sitosterolemia. Individuals with sitosterolemia exhibit hyperabsorption of β-sitosterol, as well as other sterols, and have markedly reduced secretion of sterols into the bile.

Phytosteryl/Behenyl/Octyldodecyl/Isostearyl Lauroyl Glutamate (concentration not stated) was predicted to be non-corrosive in an in vitro human skin model test. In an EpiDerm™ skin irritation assay, the irritation potential of the same ingredient (concentration not stated) was not classified as an irritant. In an in vitro cell viability assay using EpiSkin™ reconstituted human epidermis, a product containing 1% Phytosteryl/Octyldodecyl Lauroyl Glutamate was predicted to be non-irritating. The skin irritation potential of all 3 phytosteryl glutamates (each tested at 100%) was evaluated in studies using New Zealand white rabbits (groups ranging from 3 - 6 animals/study) conducted with a semi-occlusive or occlusive patch; all 3 ingredients were found to be non-irritating. A 14-d open-application skin irritation study yielded no skin reactions on 10 female guinea pigs for Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate and Phytosteryl/Octyldodecyl Lauroyl Glutamate at maximum concentrations of 100%.

In a 24-h occlusive patch test, a Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate cream (applied neat; concentration of Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate not specified) tested on 31 human subjects was deemed to be non-irritating. Two separate 24-h patch tests of Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate and Phytosteryl/Octyldodecyl Lauroyl Glutamate (both at 100%) were negative for irritation in 45 human subjects. In a human cumulative irritation patch test with 25 subjects that took place over 7 d, a face cream containing 1% Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate was determined to be non-irritating. A 7-d semi-occlusive cumulative irritation patch test with a formulation containing 1.5% Phytosteryl/Octyldodecyl Lauroyl Glutamate was performed with 38 subjects; no irritation was observed.

In a DPRA, Phytosteryl/Octyldodecyl Lauroyl Glutamate, prepared as a 100 mM stock solution and tested for cysteine and lysine depletion, was predicted to have minimal reactivity, with a mean depletion of 1.8% for cysteine and 0.1% for lysine. Three separate guinea pig maximization tests (15 female guinea pigs/test group) were negative when performed on Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate (25% intradermal induction, 100% epidermal induction, 100% challenge); Phytosteryl/Behenyl/Octyldodecyl/Isostearyl Lauroyl Glutamate (10% intradermal induction, 100% epidermal induction, 50 and 100% challenge); Phytosteryl/Octyldodecyl Lauroyl Glutamate (5% intradermal induction, 100% epidermal induction, 10% challenge). In an HRIPT, a face cream containing 5% Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate (102 subjects, tested neat, occlusive patch) was not an irritant or a sensitizer. In another HRIPT, a mixture containing 5.999% Phytosteryl/Octyldodecyl Lauroyl Glutamate (219 subjects; tested neat, occlusive patch) was not an irritant or a sensitizer.

A tissue equivalent assay was conducted to evaluate the ocular irritancy potential of a face cream containing 1% Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate; the t_{50} was > 24 h. In an EpiOcular™ in vitro assay, Phytosteryl/Behenyl/Octyldodecyl/Isostearyl Lauroyl Glutamate (concentration not stated) was found to be a non/minimal irritant. All three phytosteryl glutamates (maximum concentration 100%), each tested as a single instillation into the eyes of New Zealand

White rabbits (groups ranging from 3 - 6 animals), were negative for eye irritation. In a clinical in-use test in which a product containing 0.5% Phytosteryl/Octyldodecyl Lauroyl Glutamate was applied to the eye area and lashes (n=30), no skin or eye discomfort was reported by the subjects.

DISCUSSION

The Panel reviewed the safety of 3 phytosteryl glutamates and concluded that the available data are sufficient for determining that all 3 ingredients are safe in cosmetics in the present practices of use and concentration. The Panel noted there was a robust amount of data to support the safety of these ingredients, including negative 28-day oral toxicity and negative mutagenicity studies. The Panel noted the lack of developmental and reproductive toxicity data; however, concern for the lack of these data was mitigated because these ingredients are not expected to be absorbed. The Panel also noted the lack of confirmatory sensitization data at maximum reported concentrations of use; however, this need was mitigated by the negative guinea pig maximization assays performed on the phytosteryl glutamates that included epidermal induction and challenge at 100%

The Panel discussed that sitosterol is a component of these ingredients, and noted the possible biological effects it can cause when it interacts with different receptors in the body. In the intestine, the transporter ABCG5GA transports plant sterols from the enterocytes back into the gut. Mutations in the ABC transporters ABCG5 and ABCG8 have been shown to lead to the accumulation of plant sterols causing the disorder sitosterolemia.

The Panel expressed concern regarding heavy metals that may be present in these ingredients. They stressed that the cosmetics industry should continue to use the necessary procedures to minimize impurities in cosmetic formulations according to limits set by the FDA and EPA.

The Panel also discussed the issue of incidental inhalation exposure resulting from these ingredients (for example, Phytosteryl/Octyldodecyl Lauroyl Glutamate is used in aerosol deodorant (up to 0.1 %), and in face powders (at concentrations up to 5%)). Inhalation toxicity data were not available. However, the Panel noted that the majority of droplets/particles would not be respirable to any appreciable amount. Furthermore, droplets/particles deposited in the nasopharyngeal or tracheobronchial regions of the respiratory tract present no toxicological concerns based on the chemical and biological properties of these ingredients. Coupled with the small actual exposure in the breathing zone and the low concentrations at which these ingredients are used (or expected to be used) in potentially inhaled products, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at https://www.cir-safety.org/cir-findings.

Finally, the Panel's respiratory exposure resource document (see link above) notes that airbrush technology presents a potential safety concern, and that no data are available for consumer habits and practices thereof. As a result of deficiencies in these critical data needs, the safety of cosmetic ingredients applied by airbrush delivery systems cannot be assessed by the Panel. Therefore, the Panel has found the data insufficient to support the safe use of cosmetic ingredients applied via an airbrush delivery system.

CONCLUSION

The Expert Panel for Cosmetic Ingredient Safety concluded that the following 3 phytosteryl glutamates are safe in cosmetics in the present practices of use and concentration described in this safety assessment.

Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate
Phytosteryl/Behenyl/Octyldodecyl/Isostearyl Lauroyl Glutamate
Phytosteryl/Octyldodecyl Lauroyl Glutamate

TABLES

Table 1. Definitions and reported functions of the ingredients in this safety assessment[1]

Ingredient/CAS No.	Definition	Function(s)
Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate 245443-09-8	Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate is the mixed ester of phytosterol, behenyl alcohol, and octyldodecanol with lauroyl glutamic acid.	Skin-Conditioning Agents – Occlusive
Phytosteryl/Behenyl/Octyldodecyl/ Isostearyl Lauroyl Glutamate	Phytosteryl/Behenyl/Octyldodecyl/Isostearyl Lauroyl Glutamate is the mixed ester of phytosterols, behenyl alcohol, octyldodecanol and isostearyl alcohol with lauroyl glutamic acid.	Hair Conditioning Agents; Skin-Conditioning Agents – Emollient
Phytosteryl/Octyldodecyl Lauroyl Glutamate 220465-88-3	Phytosteryl/Octyldodecyl Lauroyl Glutamate is the mixed ester of phytosterol and octyldodecanol with lauroyl glutamic acid.	Skin-Conditioning Agents – Occlusive

Table 2. Gel permeation chromatography of Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate [8]

Peak No.	No. Avg. Molecular Weight	Weight average molecular weight	Size average molecular weight	Molecular weight at the highest peak	Degree of dispersion	Area%
1	1344	1372	1402	1389	1.021	74.2
2	746	757	768	765	1.015	13.8
3	383	396	409	388	1.034	12.0

Table 3. Frequency (2023)[10] and concentration (2021)[6] of use according to likely duration and exposure and by product category.

	Phytosteryl/Octyldodecyl Lauroyl Glutamate		Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate		Phytosteryl/Behenyl/Octyldodecyl/ Isostearyl Lauroyl Glutamate	
	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
Totals*	**327**	**0.005-25**	**49**	**NR**	**1**	**0.00028-25.6**
summarized by likely duration and exposure**						
Duration of Use						
Leave-On	*312*	*0.01-25*	*49*	*NR*	*1*	*0.03-25.6*
Rinse-Off	*15*	*0.005-2*	*NR*	*NR*	*NR*	*0.00028-1*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type*						
Eye Area	29	0.1-12	4	NR	NR	1-8.6
Incidental Ingestion	133	1-25	1	NR	1	0.1-7
Incidental Inhalation-Spray	84[a]; 40[b]	0.1-2 [a]	12 [a];10 [b]	NR	NR	0.2 [a]
Incidental Inhalation-Powder	40[b]	5; 0.01-8[c]	10 [b]	NR	NR	1; 0.03-5[c]
Dermal Contact	184	0.005-25	48	NR	NR	0.00028-25.6
Deodorant (underarm)	NR	not spray: 0.1 spray: 0.1	NR	NR	NR	NR
Hair - Non-Coloring	9	0.1-2	NR	NR	NR	0.2
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	1	NR	NR	NR	NR	NR
Mucous Membrane	133	0.005-25	1	NR	1	0.1-7
Baby Products	NR	0.3	NR	NR	NR	NR
as reported by product category						
Baby Products						
Baby Lotions/Oils/Powders/Creams	NR	0.3 (not powder)				
Eye Makeup Preparations						
Eyeliner	NR	7.5				
Eye Shadow	16	12			NR	8.6
Eye Lotion	6	2.5	4	NR	NR	1
Eye Makeup Remover	1	NR				
Other Eye Makeup Preparations	6	0.1-4.2				
Hair Preparations (non-coloring)						
Hair Conditioner	3	0.1-0.7				
Rinses (non-coloring)	2	NR				
Shampoos (non-coloring)	1	NR				
Tonics, Dressings, and Other Hair Grooming Aids	2	0.7-2			NR	0.2
Other Hair Preparations	1	NR				
Makeup Preparations						
Blushers (all types)	NR	5.4			NR	7.3
Face Powders	NR	5			NR	1
Foundations	13	2.2-3.1			NR	1
Lipstick	133	1-25	1	NR	1	0.1-7
Makeup Bases	NR	1				
Rouges	1	25	22	NR	NR	25.6
Other Makeup Preparations	4	1			NR	0.42
Manicuring Preparations (Nail)						
Other Manicuring Preparations	1	NR				
Personal Cleanliness Products						
Bath Soaps and Detergents	NR	0.005				
Deodorants (underarm)	NR	0.1 (not spray) 0.1 (aerosol)				
Skin Care Preparations						
Cleansing	6	1-2			NR	0.00028-1
Face and Neck (exc shave)	30	0.3-8 (not spray)	5	NR	NR	0.03-5 (not spray)
Body and Hand (exc shave)	10	0.01-1 (not spray)	5			
Moisturizing	79	0.1-0.5 (not spray	9	NR	NR	0.5 (not spray)
Night	3	1	3	NR		
Paste Masks (mud packs)	2	0.1				
Skin Fresheners	NR	0.1-0.5				
Other Skin Care Preparations	7	0.1-2			NR	0.5

NR – not reported

*Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.

**likely duration and exposure is derived based on product category (see Use Categorization https://www.cir-safety.org/cir-findings)

[a] It is possible these products are sprays, but it is not specified whether the reported uses are sprays.

[b] Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, therefore the information is captured in both categories

[c] It is possible these products are powders, but it is not specified whether the reported uses are powders.

Table 4. Acute toxicity studies

Test Article	Vehicle	Animals/Group	Concentration/Dose	Protocol	LD_{50}/LC_{50}/Results	Reference
DERMAL						
Phytosteryl/Octyldodecyl Lauroyl Glutamate		5 male and 5 Ffmale Rats (HAnBrl: Wist)	2000 mg/kg (2.16 ml/kg)	OECD TG 402 No other details provided.	LD_{50} > 2000 mg/kg (No death occurred)	[13]
ORAL						
Phytosteryl/Behenyl/ Octyldodecyl Lauroyl Glutamate	olive oil	5 male and 5 female mice (ICR)	0, 1000, and 2000 mg/kg	Limit test	LD_{50} > 2.0 g/kg (No death occurred in all groups)	[13]
Phytosteryl/Behenyl/ Octyldodecyl/ Isostearyl Lauroyl Glutamate	corn oil	3 females x 2 group (Wister)	2000 mg/kg	OECD TG 423 No other details provided.	LD_{50}(rat) > 2000 mg/kg (No death occurred in all groups).	[13]
Phytosteryl/Octyldodecyl Lauroyl Glutamate	PEG 300	3 male and 3 female rats (HAnBrl: Wist)	2000 mg/kg	OECD TG 423 No other details provided.	LD_{50} > 2000 mg/kg (No death occurred)	[13]

Table 5. Genotoxicity studies

Test Article	Vehicle	Concentration/Dose	Test System	Procedure	Results	Reference
IN VITRO						
Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate	acetone	0.625, 1.25, 2.5, and 5.0 µg/ml	CHL/IU cells	In a mammalian chromosome aberration test, exposure was for 6, 24, and 48 h.	Precipitation noted in all concentrations in all exposures with and without metabolic activation. Negative test.	[13]
Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate	acetone	10, 20, 39, 78, 156, 313, 625, 1250 µg/plate	*Salmonella typhimurium*: TA98, TA100, TA1535 and TA1537; *Escherichia coli*: WP2 uvrA	Bacterial reverse mutation test (Ames test)	Precipitation was noted in 1250 µg/plate with metabolic activation in all strains, and 625 and 1250 µg/plate without metabolic activation in all strains. Cytotoxicity was seen in TA1537 and TA98 with concentration 313 µg/plate without activation. Non-mutagenic	[13]
Phytosteryl/Behenyl/Octyldodecyl /Isostearyl Lauroyl Glutamate	DMSO	100, 316, 1000, 3160, and 5000 µg/plate	*S. typhimurium*: TA98, TA100, TA1535, TA1537, and TA1538 *E. coli*: WP2 uvrA	Bacterial reverse mutation test. OECD 471. No other details provided.	Non-mutagenic	[13]
Phytosteryl/Octyldodecyl Lauroyl Glutamate	acetone	33, 100, 333, 1000, 2500, and 5000 µg/plate	*S. typhimurium*: TA98, TA100, TA1535 and TA1537; *E. coli*: WP2 uvrA	Bacterial reverse mutation test. Ames test was performed with and without S-9 metabolic activation.	Non-mutagenic	[15]
Phytosteryl/Octyldodecyl Lauroyl Glutamate	0.5% acetone	2.5 - 2500 µg/ml	Chinese hamster V79 cells	An in-vitro assay was performed to assess test article's ability to induce structural chromosome aberration with and without S-9 metabolic activation. Exposure was for 4, 18, and 28 h. Recovery was between 14 – 24 h.	Non-clastogenic	[16]
Phytosteryl/Octyldodecyl Lauroyl Glutamate	acetone	9.4, 18.8, 37.5, 75.0, 78.1, 156.3, 312.5, 625.0, 1250.0, and 2500 µg/ml	Chinese hamster V79 cells	In vitro mammalian chromosome aberration test. Exposure for 4, 18, and 28 hrs	One precipitation at 300 µg/ml with S9 Non-clastogenic	[13]
Phytosteryl/Octyldodecyl Lauroyl Glutamate	NR	10, 100, 300, 900, 2700, and 5000 µg/ml	Mouse lymphoma L5178Y/TK cells	In vitro mammalian cell gene mutation test. Exposure was for 4 h in both groups, with and without metabolic activation.	Negative	[13]

* NR – not reported

Table 6. Dermal irritation and sensitization studies

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
IRRITATION					
In Vitro					
Phytosteryl/Behenyl/ Octyldodecyl/Isostearyl Lauroyl Glutamate	NR		OECD TG 431. MTT skin corrosion utilizing skin model test.	Non-corrosive	[13]
Phytosteryl/Behenyl/ Octyldodecyl/Isostearyl Lauroyl Glutamate	NR		OECD TG 439. Skin irritation test utilizing EpiDerm™ test	Not classified (i.e., not predicted to be an irritant)	[13]
Mixture containing 1 % Phytosteryl/Octyldodecyl Lauroyl Glutamate	1% mixture 150 mg ± 5 mg, applied in duplicate	2 different lots of reconstructed human epidermis (EpiSkin™)	EpiSkin™ assay. Negative and positive control were tested in triplicate. At the end of incubation an MTT test was performed. Samples were plated, biopsied, and the epidermis was separated from the collagen and transferred to tubes. Cell viability was then determined. Acceptability and expression of results followed	Mean viability greater than 50% is interpreted as being potentially non-irritant; in two samples the mean viability resulted in 81.1% and 72.4%, thus this mixture is considered potentially non-irritant.	[18]
Animal					
Phytosteryl/Behenyl/ Octyldodecyl Lauroyl Glutamate	100%	New Zealand White rabbits 6 males	A Draize test was performed with an occlusive patch that was applied for 24 h. Animals were observed at 24, 48, and 72 h and 1 wk post-treatment.	Mean irritation score 0.00 at all observation periods post-treatment. Not irritating.	[13]
Phytosteryl/Behenyl/ Octyldodecyl Lauroyl Glutamate	10, 30, and 100% (w/w) in petrolatum	Dunkin-Hartley Albino guinea pigs 10 females	An open application was made for 14 d.	No skin reactions were observed at any concentration. Not irritating.	[13]
Phytosteryl/Behenyl/ Octyldodecyl/Isostearyl Lauroyl Glutamate	100%	New Zealand White rabbits 3 males	OECD TG 404. An occlusive patch was applied for 4 h. Test sites was observed at 1, 24, 48, and 72 h after treatment.	Mean score 0.00 at all observation periods post treatment. Not irritating.	[13]
Phytosteryl/Octyldodecyl Lauroyl Glutamate	100%	New Zealand rabbits 1 male 2 females	OECD TG 404. A semi-occlusive application was applied for 4 h. Irritation level was observed for 1, 24, 48, and 72 h after treatment.	Mean score 0.00 at all observation periods post treatment. Not irritating	[13]
Phytosteryl/Octyldodecyl Lauroyl Glutamate	0, 10, 30, and 100% (w/w) in petrolatum	Dunkin-Hartley albino guinea pigs 10 females	An open application was made for 14 d.	No skin reactions were observed at any concentration. Non-irritating.	[13]
Human					
Phytosteryl/Behenyl/ Octyldodecyl/Lauroyl Glutamate cream (concentration not specified)	15 µl, appliedneat	31 subjects	24-h occlusive patch test. The test sample was applied to the backs of subjects and fixed with plaster. Reactions were scored after 30 min, and at 24 and 48 h after patch removal.	One subject had a 0.5 score after 30 min that resolved to 0 at 24 and 48 h after patch removal. Another subject had a score of 0.5 only at 48 h after patch removal. All other subjects had scores of 0 at all time points. This cream is considered a non-irritant on human skin.	[8]
Face cream containing 1% Phytosteryl/Behenyl/ Octyldodecyl Lauroyl Glutamate	0.2 ml, applied neat	25 subjects	Human cumulative irritation patch test. On study day 1, a semi-occlusive patch containing 0.2.ml of the test sample was applied to the backs of subjects for 23 (+/- 1) h. On study days 2-6, patches were removed and graded 30 min following patch removal using a 60-W daylight blue bulb. Patches were then reapplied to the same area on the subjects. On study day 7, patches were removed and graded.	On the last day of the study, 5 subjects exhibited elevated irritation grades (≥ 2). All elevated grades were resolved. Two adverse events occurred during the course of the study, but were related to study procedures (i.e., tape irritation), not the test study material. Based on the cumulative irritation index, no unexpected skin conditions were observed and the test material elicited skin responses similar to the negative irritant control.	[19]
Phytosteryl/Behenyl/ Octyldodecyl Lauroyl Glutamate	100% (w/w) (active ingredient.)	45 subjects 8 males 37 females	An occlusive patch was applied in the crooked side of the upper arm for 24 h.	Non-irritating	[13]

Table 6. Dermal irritation and sensitization studies

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
Phytosteryl/Octyldodecyl/ Lauroyl Glutamate	100% (w/w) (active ingredient)	45 subjects 8 males 37 females	An occlusive patch was applied in the crooked side of the upper arm for 24 h.	Non-irritating	[13]
Facial essence containing 1.5% Phytosteryl/Octyldodecyl Lauroyl Glutamate	0.2 ml, applied neat	38 subjects	A 7-d semi-occlusive cumulative irritation patch test was performed. Distilled water served as the negative control and 0.75% SLS served as a positive control. Prior to the first application, sites were wiped with 70% isopropyl alcohol. Two-tenths (0.2) ml of the test sample was applied with a 2cm x 2cm pad to the back and upper arm for 23 (± 1) h and then removed. After patch removal sites were evaluated, and the responses recorded. This was repeated daily for 7 d.	Under the conditions employed in the study, the subjects showed no evidence of irritation.	[20]
SENSITIZATION					
In Chemico					
Phytosteryl/Octyldodecyl Lauroyl Glutamate	Concentration not stated		A DPRA that measures the reactivity of Phytosteryl/Octyldodecyl Lauroyl Glutamate to cysteine and lysine peptides was conducted. Phytosteryl/Octyldodecyl Lauroyl Glutamate was dissolved in acetonitrile to prepare 100mM stock solution. The positive control was in the appropriate range for both peptides cysteine 60.8% < mean <100%; lysine: 40.2 < mean < 69.4%)	The percent peptide depletion value of cysteine was 1.8% and 0.1% for lysine. Depletion less than 14.9 is considered to have no, or minimal reactivity, and is predicted to be negative for dermal sensitization. The control had the expected results and was predicted to have minimal reactivity.	[21]
Animal					
Phytosteryl/Behenyl/ Octyldodecyl Lauroyl Glutamate	Intradermal induction 25% (in olive oil) Epidermal induction 100% Challenge 100%	15 Female Dunkin-Hartley Albino guinea pigs 10 test group 5 control	A guinea pig maximization test was performed. No reactions were observed at 24 and 48 h after removal of the patch.	Negative	[13]
Phytosteryl/Behenyl/ Octyldodecyl/Isostearyl Lauroyl Glutamate	Intradermal induction 10% (in liquid paraffin) Epidermal induction 100% Challenge 50, 100% (PEG 300, vehicle)	15 Female Hartley Albino guinea pigs 10 test group 5 control	OECD 406. A guinea pig maximization test was performed. No reactions observed 24 and 48 h after removal of patch.	Negative	[13]
Phytosteryl/Octyldodecyl Lauroyl Glutamate	Intradermal induction 5% (in PEG 400) Epidermal induction 100% Challenge 10% (in PEG 400)	15 female Himalayan spotted guinea pigs 10 test group 5 control	A guinea pig maximization test was performed. No reactions were observed at 24 and 48 h after removal of the patch.	Negative	[13]

Table 6. Dermal irritation and sensitization studies

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
			Human		
Face cream containing 5% Phytosteryl/Behenyl/ Octyldodecyl Lauroyl Glutamate	0.2 ml applied neat	102 subjects	HRIPT evaluating sensitization potential. During induction, product was placed on an occlusive patch (2 cm x 2 cm) no longer than 15 min prior to patch application. The induction phase consisted of nine 24-h applications made over 3 wk. After a 10–15-d non-treatment period, challenge patches were applied for 24 h to previously untreated sites. Reactions were scored at 48 h and 72 h after patch removal.	During induction, no reactions were reported, and none were observed for any of the subjects at challenge. Under the conditions employed in this study, there was no evidence of sensitization to the product.	[22]
Mixture containing 5.999% Phytosteryl/Octyldodecyl Lauroyl Glutamate	0.2ml applied as supplied.	219 subjects	HRIPT evaluating sensitization potential. During induction, the product was placed on an occlusive patch (2 cm x 2 cm), which was applied to the infrascapular area of the back (either to right or left of midline), or to the upper arm. Induction phase consisted of nine 24-h applications made over 4 consecutive weeks. After a 10-15 d non-treatment period, challenge patches were applied for 24 h to previously untreated sites. Reactions were scored at 48 h and 72 h after patch removal.	During induction, no reactions were reported, and none were observed for any of the subjects at challenge. Under the conditions employed in this study, there was no evidence of sensitization to the product.	[23]

Abbreviations: HRIPT – human repeated insult patch test; SIOPT – single insult occlusive patch test; SLS – sodium lauryl sulfate

Table 7. Ocular irritation studies

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
			IN VITRO			
A face cream containing 1% Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate	none	100 µl undiluted and incubated.		The conversion of 3-[4,5,-dimethylthiazol-2-y1]-2,5-diphenyltetrazolium bromide (MTT) by EpiOcular™ cultures, was performed.	MTT was not reduced in the absence of viable tissue; the t_{50} (duration of exposure resulting in a 50% decrease in MTT conversion) was > 24 h.	[24]
Phytosteryl/Behenyl/Octyldodecyl/ Isostearyl Lauroyl Glutamate	NR	NR		in vitro EpiOcular™ eye irritation study	Non/minimal irritant	[13]
			ANIMAL			
Phytosteryl/Behenyl/ Octyldodecyl Lauroyl Glutamate	olive oil	10% (W/W) (A.I)	6 male New Zealand White rabbits	Draize test	Non-irritating. At 24, 48, 72, and 96 h, the mean scores were 0.0.	[13]
Phytosteryl/Behenyl/Octyldodecyl/ Isostearyl Lauroyl Glutamate	none	100%	3 male New Zealand White rabbits	OECD TG 405	Not irritating to rabbit eye. Irritation to the cornea and iris were 0.0 at 1, 24, 48, and 72 h. Conjunctiva redness at 1 and 24 h was 1.0; after 48 and 72 h, redness was 0.0. Conjunctiva chemosis after 1 h was 0.3; after 24, 48 and 72 h, chemosis was 0.0.	[13]
Phytosteryl/Octyldodecyl Lauroyl Glutamate	none	100%	1 male and 2 female New Zealand White rabbits	OECD TG 405	Not irritating to rabbit eye. After 1 h, mean score of 1.00. After 24 – 72 h, a mean score of 0.00	[13]

*W/W – weight for weight

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