# Safety Assessment of Phytosteryl Glutamates as Used in Cosmetics

Status: Draft Final Report for Panel Review
Release Date: May 19, 2023
Panel Meeting Date: June 12-13, 2023

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Thomas J. Slaga, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. Previous Panel members involved in this assessment: Daniel C. Liebler, Ph.D., and Ronald C. Shank, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D., and the Senior Director is Monice Fiume. This report was prepared by Wilbur Johnson, Jr., M.S., former Senior Scientific Analyst/Writer, and Regina Tucker, M.S., Scientific Analyst/Writer, CIR.


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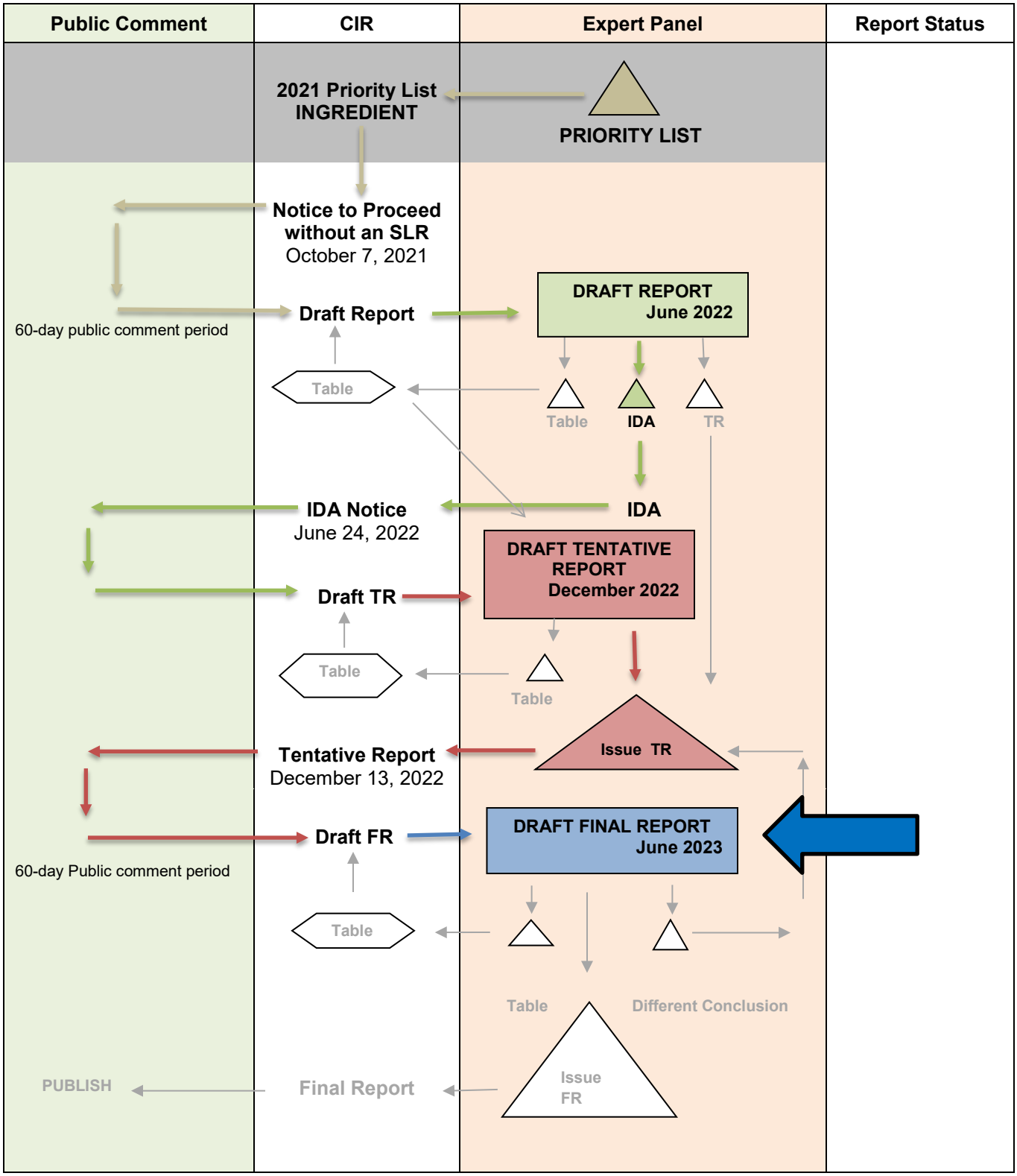
# SAFETY ASSESSMENT FLOW CHART

**INGREDIENT/FAMILY** Phytosteryl Glutamates

**MEETING** June 2023



Cosmetic Ingredient Review

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**Memorandum**

**To:** Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
**From**: Regina Tucker, M.S., Scientific Analyst/Writer, CIR
**Date:** May 19, 2023
**Subject:** Safety Assessment of Phytosteryl Glutamates as Used in Cosmetics

Enclosed is the Draft Final Report on the Safety Assessment of Phytosteryl Glutamates as Used in Cosmetics. (It is identified in the report package as *report_PhytosterylGlutamates_062023*.) At the December 2022 meeting, the Expert Panel for Cosmetic Ingredient Safety (Panel) issued a Tentative Report for public comment with the conclusion that the available data are insufficient to make a determination that the 3 phytosteryl glutamates are safe under the intended conditions of use in cosmetic formulations.

In order to come to a conclusion of safety for these cosmetic ingredients, the following additional data are needed:

- Method of manufacture
- Impurities data
- 28-day dermal toxicity
    - If positive, other toxicological endpoints, such as developmental and reproductive toxicity, genotoxicity, and carcinogenicity data, may be needed.
- Irritation and sensitization data at maximum reported concentration of use.
- Ocular irritation data, if available

Since the issuing of the Tentative Report, published data on plant sterols and sitosterolemia, 2023 VCRP data, and the following unpublished data submitted by the Council have been incorporated into the Draft Final Report, as indicated by highlighting:

- Manufacturing methods for each ingredient *(data1_PhytosterylGlutamates_062023)*
- Safety data-Toxicological summary table *(data2_PhytosterylGlutamates_062023)*

The Panel should review comments on the Tentative Report submitted by the CIR Science and Support Committee (SSC) regarding the request for a dermal 28-d study, in light of having a negative oral 28-d study *(SSCcomments_PhytosterylGlutamates_062023)*. Comments on the Tentative Report provided by the Council (*PCPCcomments_PhytosterylGlutamates_062023*) are also included and were addressed, as indicated in the responses to these comments (*response-PCPCcomments_PhytosterylGlutamtes_062023*).

Also included in this package for your review are the report history (*history_PhytosterylGlutamates_062023*), flow chart (*flow_PhytosterylGlutamates_062023*), literature search strategy (*search_PhytosterylGlutamates_062023*), ingredient data profile (*dataprofile_PhytosterylGlutamates_062023*), and transcripts from previous meetings (*transcripts_PhytosterylGlutamates_062023*).

The Panel should carefully consider the Abstract, Discussion, and Conclusion presented in this report. If these are satisfactory, the Panel should issue a Final Report. However, if the submitted data resolve the needs identified above, the Panel should reconsider the Discussion and Conclusion and issue a revised Tentative Report.

Personal Care Products Council
Committed to Safety, Quality & Innovation

**Memorandum**

**TO:** Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

**FROM:** Alexandra Kowcz, MS, MBA
Industry Liaison to the CIR Expert Panel

**DATE:** January 19, 2023

**SUBJECT:** Tentative Report: Safety Assessment of Phytosteryl Glutamates as Used in Cosmetics (release date: December 13, 2022)

The Personal Care Products Council respectfully submits the following comments on the tentative report, Safety Assessment of Phytosteryl Glutamates as Used in Cosmetics.

Key Issues

The technical name for Phytosteryl/Octyldodecyl Lauroyl Glutamate, L-Glutamic acid, N-(1-oxododecyl)-, mixed (3β, 24R)-ergost-5-en-3-yl and 2-octyldodecyl and (3β, 22E)-stigmasta-5,22-dien-3-yl and (3β)-stigmast-5-en-3-yl esters (listed in the Dictionary and associated with the CAS number), should be added to the CIR report. This name indicates that the phytosterol component of this ingredient is known. Based on Pubchem searches, they are campesterol, stigmasterol and β-sitosterol. This should be made clear in the CIR report.

A CAS number (245443-09-8) has also been identified for Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate which has been added to the Dictionary. This CAS number is associated with the technical name, L-Glutamic acid, N-(1-oxododecyl)-, mixed docosyl (3.beta.,24R)-ergost-5-en-3-yl 2-octyldodecyl (3.beta.,22E)-stigmasta-5,22-dien-3-yl(3.beta.)-stigmast-5-en-3-yl esters, which has also been added to the Dictionary. This ingredient includes the same phytosterol components as identified for Phytosteryl/Octyldodecyl Lauroyl Glutamate.

Introduction – In the Introduction, it should be made clear that the CIR report on phytosterols also included phytosteryl alkanoates, such as Phytosteryl Isostearate. The Introduction should also note that CIR has reviewed the safety of Octyldodecanol as used in cosmetics and found it safe as used.

Additional Considerations

Introduction – Although Lauroyl Glutamic Acid was not in use at the time of the original CIR report, the Introduction should note that Sodium Lauroyl Glutamate was in use (maximum use concentration up to 40% in skin cleansing products).

Definition and Structure – The CAS number needs to be corrected in this section (it ends with "-33", it should end with "-3").

Chemical Properties – In the text, please indicate that gel permeation chromatography indicated that three peaks were identified in Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate.

Cosmetic Use – Please revise: "ingredients are used in products that are reported to be used in formulations that could be incidentally ingested" ("are used in products that" should be deleted).

Genotoxicity – Please revise "a positive increase" (delete "positive")

Other Relevant Studies – This section should be deleted as it refers to a *Rosa centifolia*-derived ingredient rather than the ingredients in this report.

Dermal Irritation and Sensitization; Summary – In the description of the DPRA, the reactivity of Phytosteryl/Octyldodecyl Lauroyl Glutamate is being studied. Rather than stating: "Both peptides showed minimal reactivity." It would be clearer to state that "Phytosteryl/Octyldodecyl Lauroyl Glutamate showed little reactivity to both peptides."

Ocular Irritation; Summary – Although more than one test article may have been studied in the EpiOcular study, only one test article, Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate is relevant to this report. Please revise: "Approximately 100 µl of each test article" to "Approximately 100 µl of the test article".

Clinical Studies; Summary – Based on Dr. Klaassen's comments during the December 2022 meeting, 0.49995% should be rounded to 0.5%.

Summary – Please state the FDA product category in which the 25.6% concentration was reported (rouges).

Summary; Table 4 – In the 24-hour patch test, Phytosteryl/Behenyl/Octyldodecyl Lauroyl (Glutamate needs to added to the Summary) was tested undiluted; "(concentration not stated)" should be deleted.

Table 4 – In the description of the DPRA, "acetonitrile" does not belong in the Concentration/Dose column. It would be clearer if the description of the study indicated that the assay measures the reactivity of Phytosteryl/Octyldodecyl Lauroyl Glutamate to cysteine and lysine peptides.

**Draft Report Comment Responses**

| Phytosteryl Glutamates – March 2023-Regina Tucker | |
|---|---|
| **Comment Submitter: Personal Care Products Council**<br>**Date of Submission: January 19, 2023** | |
| **Comment** | **Response/Action** |
| The technical name for Phytosteryl/Octyldodecyl Lauroyl Glutamate, L-Glutamic acid, N-(1- oxododecyl)-, mixed (3β, 24R)-ergost-5-en-3-yl and 2-octyldodecyl and (3β, 22E)-stigmasta5,22-dien-3-yl and (3β)-stigmast-5-en-3-yl esters (listed in the Dictionary and associated with the CAS number), should be added to the CIR report. This name indicates that the phytosterol component of this ingredient is known. Based on Pubchem searches, they are campesterol, stigmasterol and β-sitosterol. This should be made clear in the CIR report. | Components are provided in the chemistry section of the report. |
| A CAS number (245443-09-8) has also been identified for Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate which has been added to the Dictionary. This CAS number is associated with the technical name, L-Glutamic acid, N-(1-oxododecyl)-, mixed docosyl (3.beta.,24R)-ergost-5- en-3-yl 2-octyldodecyl (3.beta.,22E)-stigmasta-5,22-dien-3-yl(3.beta.)-stigmast-5-en-3-yl esters, which has also been added to the Dictionary. This ingredient includes the same phytosterol components as identified for Phytosteryl/Octyldodecyl Lauroyl Glutamate | CAS number has been updated. |
| Introduction – In the Introduction, it should be made clear that the CIR report on phytosterols also included phytosteryl alkanoates, such as Phytosteryl Isostearate. The Introduction should also note that CIR has reviewed the safety of Octyldodecanol as used in cosmetics and found it safe as used. | Addressed |
| Introduction – Although Lauroyl Glutamic Acid was not in use at the time of the original CIR report, the Introduction should note that Sodium Lauroyl Glutamate was in use (maximum use concentration up to 40% in skin cleansing products) | Addressed |
| Definition and Structure – The CAS number needs to be corrected in this section (it ends with "- 33", it should end with "-3") | Addressed |
| Chemical Properties – In the text, please indicate that gel permeation chromatography indicated that three peaks were identified in Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate. | This information has been omitted after discussion by the Panel. |
| Cosmetic Use – Please revise: "ingredients are used in products that are reported to be used in formulations that could be incidentally ingested" ("are used in products that" should be deleted). | Addressed |
| Genotoxicity – Please revise "a positive increase" (delete "positive") | Addressed |
| Other Relevant Studies – This section should be deleted as it refers to a Rosa centifolia-derived ingredient rather than the ingredients in this report. | Addressed |
| Dermal Irritation and Sensitization; Summary – In the description of the DPRA, the reactivity of Phytosteryl/Octyldodecyl Lauroyl Glutamate is being studied. Rather than stating: "Both peptides showed minimal reactivity." It would be clearer to state that | Addressed |

| | |
|---|---|
| "Phytosteryl/Octyldodecyl Lauroyl Glutamate showed little reactivity to both peptides." | |
| Ocular Irritation; Summary – Although more than one test article may have been studied in the EpiOcular study, only one test article, Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate is relevant to this report. Please revise: "Approximately 100 µl of each test article" to "Approximately 100 µl of the test article". | Addressed |
| Clinical Studies; Summary – Based on Dr. Klaassen's comments during the December 2022 meeting, 0.49995% should be rounded to 0.5%. | Addressed |
| Summary – Please state the FDA product category in which the 25.6% concentration was reported (rouges) | Addressed |
| Summary; Table 4 – In the 24-hour patch test, Phytosteryl/Behenyl/Octyldodecyl Lauroyl (Glutamate needs to added to the Summary) was tested undiluted; "(concentration not stated)" should be deleted. | Addressed |
| Table 4 – In the description of the DPRA, "acetonitrile" does not belong in the Concentration/Dose column. It would be clearer if the description of the study indicated that the assay measures the reactivity of Phytosteryl/Octyldodecyl Lauroyl Glutamate to cysteine and lysine peptides | Addressed |

Personal Care Products Council
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**TO:** Bart Heldreth Ph.D., Executive Director – Cosmetic Ingredient Review
Expert Panel for Cosmetic Ingredient Safety

**FROM:** CIR Science and Support Committee of the Personal Care Products Council

**DATE:** February 10, 2023

**SUBJECT:** Tentative Report: Safety Assessment of Phytosteryl Glutamates as Used in Cosmetics

The CIR Science and Support Committee (CIR SSC) appreciates the opportunity to comment on the tentative report, Safety Assessment of Phytosteryl Glutamates as Used in Cosmetics.

We question the request for a dermal 28-day study when a guideline 28-day oral study on Phytosteryl/Octyldodecyl Lauroyl Glutamate showing no signals is available. In general, oral studies are expected to result in higher systemic exposure and in more conservative estimates of systemic toxicity relative to dermal exposure studies. These ingredients consist of components that all have been previously reviewed by CIR and found safe for use, and these components are all found in the diet. If the concern is the potential for the phytosterol components to cause endocrine effects, the information in the CIR report on phytosterols and phytosteryl alkanoates should be summarized in this report.

# Phytosteryl Glutamates-History

## October 2021

A Scientific Literature Review (SLR) Notice to Proceed was issued and the following data was requested:

- Chemistry information, including composition and structure, method of manufacture, and impurities data (including residual monomer content)
- Toxicokinetic data relevant to routes of exposure expected with cosmetic use
- Short-term, subchronic, and chronic dermal/oral toxicity data
- Developmental and reproductive toxicity data
- Genotoxicity data
- Carcinogenicity data
- Dermal irritation and sensitization data at maximum reported use concentrations
- Inhalation toxicity data; and
- Any other relevant safety information that may be available

The following unpublished data was received:

Summary data received for Phytosteryl/Octyldodecyl Lauroyl Glutamate:

- Repeated insult patch test mixture containing 5.999% Phytosteryl/Octyldodecyl Lauroyl Glutamate.
- Primary cutaneous tolerance: Cytotoxicity study performed on an EPISKIN® reconstructed human epidermis model (test mixture containing 1% Phytosteryl/Octyldodecyl Lauroyl Glutamate).

## April 2022

The following unpublished data, all received from the Council, have been added to the draft report and are included for the Panel's review:

- Updated (2022) VCRP data
- Human skin irritation study on an epidermis model containing 1% Phytosteryl/Octyldodecyl Lauroyl Glutamate)
- Skin sensitization study (HRIPT) on a test mixture containing 5.999% Phytosteryl/Octyldodecyl Lauroyl Glutamate)

## June 2022

The Panel issues an Insufficient Data Announcement, with the following data needs:

The additional data needed to determine safety for these cosmetic ingredients and address data insufficiencies include:

- Method of manufacturing
- Impurities
- Dermal toxicity (28-day dermal)
  If positive, other toxicological endpoints (e.g., developmental and reproductive toxicity, genotoxicity, carcinogenicity, etc.)
- Oral toxicity
- Sensitization and Irritation up 25% for Phytosteryl/Octyldodecyl Lauroyl Glutamate
- Ocular irritation

## December 2022

The following unpublished data were received:

- *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay
- In vitro chromosome aberration assay in Chinese hamster V79 cells with Phytosteryl Octyldodecyl Lauroyl Glutamate
- 28-Day oral toxicity (gavage) study in the Wistar rat (Phytosteryl/Octyldodecyl Lauroyl Glutamate).
- Direct peptide reactivity assay data for L-glutamic acid, N-(1-oxododecyl), mixed (3.Beta., 24R)-ergost-5-en-3yl and 2-octyldodecyl and (3.beta.,22E)- stigmasta-5,220dien-3-yl and (3.beta.)-stigmast-5-en-3-yl esters (Phytosteryl Octyldodecyl Lauroyl Glutamate).
- Human cumulative irritation patch test (facial essence with 1.5% Phytosteryl/Octyldodecyl Lauroyl Glutamate)
- Human repeated insult patch test face cream containing 5% Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate.

- Tissue equivalent assay with Epiocular™ cultures (1% Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate in a face cream).
- Human cumulative irritation patch test ((1% Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate in a face cream
- Summary information – Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate.

The Panel issues a Tentative Report for public comment and concluded the available data are insufficient to make a determination of safety for the following 3 phytosterol glutamates: Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate Phytosteryl/Behenyl/ Octyldodecyl/Isostearyl Lauroyl Glutamate Phytosteryl/Octyldodecyl Lauroyl Glutamate

The additional data needed to determine safety for these cosmetic ingredients and address data insufficiencies include:

- Method of manufacturing
- Impurities
- 28-day dermal toxicity
  - If positive, other toxicological endpoints (e.g., DART, genotoxicity, carcinogenicity, etc.) may be needed
- Sensitization and irritation data at maximum reported use concentrations
- Ocular irritation if available

**June 2023**

The following unpublished data, all received from the Council, have been added to the draft final report and are included for the Panel's review:

- Manufacturing methods for each ingredient
- Safety data – Toxicological summary table

In addition to the aforementioned unpublished data, published data on sterol transporters ABCG5 and ABCGE were added to the report, and comments from the Personal Care Products Council and CIR Science and Support Committee were also received.

| ***Phytosteryl Glutamates* Ingredients Data Profile* –June 2023 – Wilbur Johnson/Regina Tucker** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | **Toxico-kinetics** | | **Acute Tox** | | | **Repeated Dose Tox** | | | **DART** | | **Genotox** | | **Carci** | | **Dermal Irritation** | | | **Dermal Sensitization** | | | | **Ocular Irritation** | | **Clinical Studies** | |
| | **Reported Use** | **GRAS** | **Method of Mfg** | **Constituents** | **Impurities** | **Dermal Penetration** | **ADME** | **Dermal** | **Oral** | **Inhalation** | **Dermal** | **Oral** | **Inhalation** | **Dermal** | **Oral** | **In Vitro** | **In Vivo** | **Dermal** | **Oral** | **In Vitro** | **Animal** | **Human** | **In Vitro** | **Animal** | **Human** | **Phototoxicity** | **In Vitro** | **Animal** | **Case Report** | **Other Clinical Reports** |
| Phytosteryl/Octyldodecyl Lauroyl Glutamate | X | | X | | X | | | X | X | | | X | | | | X | X | | | X | X | X | X | X | X | | | X | | X |
| Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate | X | | X | X | X | | | | X | | | | | | | X | | | | | X | X | | X | X | | X | X | | |
| Phytosteryl/Behenyl/Octyldodecyl/ Isostearyl Lauroyl Glutamate | X | | X | | X | | | | X | | | | | | | X | | | | X | X | | | X | | | | X | | |

* "X" indicates that data were available in a category for the ingredient

# Phytosteryl Glutamates

| Ingredient | CAS # | InfoBase | SciFinder | PubMed | | FDA | EU | ECHA | IUCLID | SIDS | HPVIS | NICNAS | NTIS | NTP | WHO | FAO | ECE-TOC | Web |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Phytosteryl/Octyldodecyl Lauroyl Glutamate | 220465-88-3 | Yes | | 0/0 | | No | No | No | No | No | No | No | No | No | No | No | No | Yes |
| Phytosteryl/Behenyl/Octyldodecyl /Isostearyl Lauroyl Glutamate | No CAS No. | Yes | | 0/0 | | No | No | No | No | No | No | No | No | No | No | No | No | Yes |
| Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate | 245443-09-8 | Yes | | 0/0 | | No | No | No | No | No | No | No | No | No | No | No | No | Yes |

## Search Strategy

[*document search strategy used for SciFinder, PubMed, and Toxnet*]

[*identify total # of hits /# hits that were useful or examined for usefulness*]

## LINKS

InfoBase (self-reminder that this info has been accessed; not a public website) - http://www.personalcarecouncil.org/science-safety/line-infobase
ScfFinder (usually a combined search for all ingredients in report; list # of this/# useful) - https://scifinder.cas.org/scifinder
PubMed (usually a combined search for all ingredients in report; list # of this/# useful) - http://www.ncbi.nlm.nih.gov/pubmed
Toxnet databases (usually a combined search for all ingredients in report; list # of this/# useful) – https://toxnet.nlm.nih.gov/ (includes Toxline; HSDB; ChemIDPlus; DAR; IRIS; CCRIS; CPDB; GENE-TOX)

FDA databases – http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm (CFR); then,
list of all databases: http://www.fda.gov/ForIndustry/FDABasicsforIndustry/ucm234631.htm; then,
https://www.fda.gov/food/food-additives-petitions/substances-added-food-formerly-eafus (Substances added to Food);
http://www.fda.gov/food/ingredientspackaginglabeling/gras/default.htm (GRAS);
https://www.fda.gov/food/generally-recognized-safe-gras/gras-substances-scogs-database (SCOGS database);
http://www.accessdata.fda.gov/scripts/fdcc/?set=IndirectAdditives (indirect food additives list);
http://www.fda.gov/Drugs/InformationOnDrugs/default.htm (drug approvals and database);
http://www.fda.gov/downloads/AboutFDA/CentersOffices/CDER/UCM135688.pdf (OTC ingredient list);
http://www.accessdata.fda.gov/scripts/cder/iig/ (inactive ingredients approved for drugs)

EU (European Union); check CosIng (cosmetic ingredient database) for restrictions and SCCS (Scientific Committee for Consumer Safety) opinions - http://ec.europa.eu/growth/tools-databases/cosing/

ECHA (European Chemicals Agency – REACH dossiers) – http://echa.europa.eu/information-on-chemicals;jsessionid=A978100B4E4CC39C78C93A851EB3E3C7.live1
IUCLID (International Uniform Chemical Information Database) - https://iuclid6.echa.europa.eu/search
OECD SIDS documents (Organisation for Economic Co-operation and Development Screening Info Data Sets)- http://webnet.oecd.org/hpv/ui/Search.aspx
HPVIS (EPA High-Production Volume Info Systems) - https://ofmext.epa.gov/hpvis/HPVISlogon
NICNAS (Australian National Industrial Chemical Notification and Assessment Scheme)- https://www.industrialchemicals.gov.au/chemical-information/search-assessments?assessmentcasnumber=39346-84-4

NTIS (National Technical Information Service) - http://www.ntis.gov/
NTP (National Toxicology Program ) - http://ntp.niehs.nih.gov/
WHO (World Health Organization) technical reports - http://www.who.int/biologicals/technical_report_series/en/
FAO (Food and Agriculture Organization of the United Nations) - http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/ (FAO);
FEMA (Flavor & Extract Manufacturers Association) - http://www.femaflavor.org/search/apachesolr_search/
Web – perform general search; may find technical data sheets, published reports, etc
ECETOC (European Center for Ecotoxicology and Toxicology Database) - http://www.ecetoc.org/

**JUNE 2022 PANEL MEETING – INITIAL REVIEW/DRAFT REPORT**

**Belsito's Team Meeting – June 16, 2022**

**Dr. Belsito** - Yeah. Okie doke. And then the last one we have or is phytosteryl glutamates?

**Dr. Rettie** - So this is brand new.

**Dr. Snyder** - Yes.

**Dr. Belsito** - So the first question that I had is, have we reviewed any constituents that we can read across like fatty alcohols, moral glutamic acid phytosterols? I mean, I think we have no. Then there's some read across material we can bring in.

**Monice Fiume (CIR)** - So I believe I'm PDF page 10 in the intro. It does say some of the reports that have been reviewed previously. Are they useful?

**Dr. Belsito** - Yeah, I mean, that's what I mean. Can't we bring the way to in?

**Monice Fiume (CIR)** - OK.

**Dr. Belsito** - Because it's not been brought into this report.

**Monice Fiume (CIR)** - So Don, that's going to be up to the panel. Typically we refer to them and then provide the link that those reports are available unless those data are complete, read across and you want them brought into the report. Typically, we've been referring to them. We haven't brought the data in in a while, but whatever the panel prefers for use in the report is what we can do.

**Dr. Belsito** - OK. Well, I mean at this point, I thought it was insufficient for manufacturing and impurities. 28 day dermal and if absorbed, other endpoints such as DART and genotoxicity. Oral toxicity since it's used in lipsticks up to 20 percent, 25%. And sensitization or irritation up to 25%. And ocular irritation if available and I looks to me like I made a note that it was phytosteryl octadecyl glutamate, which was the one that was used and lipsticks. Is that correct?

At a very high concentration. 25% mucus membrane. Yeah. So I wanted sensitization and irritation, particularly on that one the fitsterlate-----. But I'm open to what other people might suggest.

**Dr. Snyder** - But we don't have any absorption, distribution, metabolism, excretion data at all.

**Dr. Belsito** - That's what I said, 28 dermal. So I said insufficient manufacturing impurities 28 day dermal if absorbed other endpoints such as DART and genotoxicity. Oral tox, because it's used in lipsticks up to 25%. And sensitization and irritation on phytosteryl octadecyl glutamate at 25%, and ocular irritation if available.

**Dr. Snyder** - Agree.

**Dr. Rettie** - Yeah, there's some tox testing, but it's only a 5% and the maximal concentration is much higher as you mentioned.

**Dr. Belsito** - Yeah. Right.

**Dr. Klaassen** - There's basically no data.

**Dr. Belsito** - I'm sorry I missed that. Right.

**Dr. Klaassen** - In essence this. This document basically contains no data.

**Dr. Belsito** - Right.

**Dr. Klaassen** - Get right down to it.

**Dr. Liebler** - Right, it's. Closest thing to an empty report we've ever gotten since I've been on the panel. So these just a couple of comments on some of the things that we talked about, the idea of using, prior reviewed ingredients like the phytosterols, for example. You know the phytosterols as read across. Like Laurel oil glutamate, for example, is read across. I'm on the RIFM panel we actually do something similar to this. Where we for systemic endpoints where it's going to be, you know, often oral administration and we're interested in repeat dose tox or repro and developmental. We have what we call a Tier 3 read across, which is if we have safety data for the ingredients of an Ester that would be hydrolyzed, for example, for the components of the Esther, and we have safety data on those, we can use a that sort of Tier 3 read across to clear the endpoint. We never use Tier 3 or practically never, I think for like skin sensitization for example. But we do for the systemic endpoints. We have no history of doing that kind of

read across with CIR, I mean honestly, CIR reviews have just not really developed the read across concept nearly as much as we have on the RIFM panel, so we don't have any way to just sort of jump in and start doing that. But that is I just want to mention that is something that could be considered how on the other hand, this is an empty report with practically no information and we should have information before we try and clear these by, you know, some kind of a Tier 3 read across. So I agree with an insufficient data analysis and seeing what we get out of these. You know the components are essentially innocuous, but you know we just have no data on the on the ingredients chemical properties, method of manufacture and purities et cetera.

**Dr. Belsito** - I mean, this is the first pass. We were virtually got nothing. So we've created a list and you know we can you know I guess, if I understand you correctly, my Monice? We're not going to bring in data from the other report. They'll just be links to those reports for us to look at. The data. Is that correct?

**Monice Fiume (CIR)** - So typically that's how we've done it. And Dan, actually, I'm trying to see which report it was in the past. We would create a table of reports that were done previously that might have relevant or like we wouldn't have called it a Tier 3 read across, but that type of use and we would have included the name of the ingredient in all of the data. Just a brief summary of what was seen. Once we started adding the link to the existing reports, we've sort of gotten away from including that table, but if that's what's helpful for this report and it's useful, we can bring it in in whatever format you would like to see it.

**Dr. Klaassen** - I think it would be helpful.

**Dr. Liebler** - Yeah. I think that this is a this is something that probably ought to be considered by the CIR panel, and by the time you've gone around with an insufficient data analysis and then you've got a, you know you've got whatever response back and then your face with the question of do we use data from reports on the component ingredients for these chemicals, you know, I'll be off the panel and you guys will be considering it. But I just wanted to point out that Tier 3 approach that the RIFM panel uses, it's easier to do that when you have a single chemical to single chemical read across situation think it's one of the you know, in the RIFM we review one target chemical and it might be an Ester. So we can do a tier three with the acid and the alcohol components of the Ester and it's very straightforward. With CIR, I think one of the barriers to read across has been we're trying to review a family of related chemicals and we may have some prior data on one component of a component of that family you know. And it it's just not something we've done. But on the other hand, you know, the principles and practice of doing it are there, we've even got one paper published in chemical research in toxicology on the grouping and which does talk about read across a little bit and then another manuscript that's heading for publication on doing this multi tiered read across for different endpoints. So I mean I think that the basic we're doing this will become better established in the literature and I think that CIR should look at incorporating that in some way in. As we go forward, it might not arrive in time for this report, but let's see what data we get.

**Dr. Klaassen** - Have we ever, in regard to this report, have we ever had a report on a number of these steroids that are in these molecules like beta, sitosterol and stigmasterol? I assume we probably have not.

**Dr. Belsito** - I think we've done that no? Didn't we do Phytosterol?

**Monice Fiume (CIR)** - We have the Phytosterol report.

**Dr. Belsito** - Yeah.

**Dr. Rettie** - Yeah.

**Bart Heldreth (CIR)** - Yes.

**Dr. Klaassen** - Just as a big Phytosterol report.

**Dr. Belsito** - Yes.

**Monice Fiume (CIR)** – I think it has individual ingredients in it let me bring it up.

**Bart Heldreth (CIR)** - Yeah, the Phytosterol report was very similar in the composition of which a steroid like molecules, where the where conscribed on there. So Sigma stereo and the beta stereo is all part of those mixtures in the phytosterols report.

**Dr. Klaassen** - I mean, the sittersterile. It's toxic to some people. And the reason why it is that some people don't have the transporter that transports it back into the gut.

**Dr. Liebler** - Ah.

**Dr. Klaassen** - So prevents it's absorption, but anyhow.

**Dr. Liebler** - Interesting.

**Dr. Klaassen** - That's just a little. And some people, there's a disease called sitosterol lomel. Because they can't they absorb more than the rest of us. You know, read across Dan. You know when we're talking about different fatty acids and lengths of fatty acids. Etcetera. Doesn't bother me. But steroids, for example, like these molecules, you know, there are so many endogenous chemicals and drugs that are derived from the steroid molecule that it scares me a little bit to do too much, read across on steroid type chemicals. Do you think I'm too conservative in that?

**Dr. Liebler** - No, I mean your comment in the context of the transporter for those of you who aren't aware, I mean Curt is probably the world's authority on toxicity mechanisms involving transporter biology and it's an interesting point well taken and I'm sure you could give us chapter and verse on other examples like this. Having said that about the transporters and asteroid steriles, I think, the whole idea read across is that in general concept it can seem real scary and dicey, but read across should always be framed as a specific structure to structure comparison in the context of a particular endpoint. Where the potential risks and benefits of doing the read across can be examined and justified or rejected. And so I would say read across should not be dismissed of preemptively, but if we're going to do it, it's really only valid as a chemical to chemical comparison. Where you got a chemical that you know has an appropriate data set for an endpoint, you've got a target chemical that doesn't have the data. The idea of reading across then would be only in the context of that specific endpoint and the other mitigating factors that could you know influence the interpretation need to be at least reasonably well understood. So like read across for repeat dose toxicity you know for you know compounds that might differ in chain length or you know with a double bond here or there that can be pretty easily assessed. Repro and developmental is usually a lot dicier for read across and then you know examples like these you know, with these, phytosterols, it depends on the endpoint. So I think your concerns well taking Curt, but I don't think it should necessarily be rejected out of hand. Until you're presented with a situation where you've got to a, you know, a specific structure, you need data for and you've got a candidate, then you can look at it and really compare the chemistry. What might be known about the pharmacology, et cetera, and then make a decision on whether you would accept the read across or not.

**Dr. Belsito** - Yeah. I mean to Dan's point that at on fragrance panel will oftentimes have three or four different reader cross materials for different endpoints. Simply in many cases, because there's not one single material that provides us for read across our all of those endpoints. And then typically we are reading across, there's a justification table at the end of the report that indicates what we're using to read across for and the rationale behind using that.

**Dr. Klaassen** - So at on as a as a medicinal chemist, does read across work great in pharmacology?

**Dr. Liebler** - I don't think it's ever used. There's no needs. There's no reason to do it.

**Dr. Klaassen** - Because it doesn't work in pharmacology.

**Dr. Liebler** - Well. It's not used because it's not needed or necessary or sufficient. I mean it's read across is used in the assessment of toxicity data for an endpoint.

**Dr. Klaassen** - For when you don't have data.

**Dr. Liebler** - Right.

**Dr. Belsito** - I mean, I think we can just try and look at it. You know what I mean. We have our data needs manufacturing and impurities. We're not going to be able to read across from. 28 day dermal. We may be, I don't know. Oral tox. We're going to have an issue cause of the high levels and lipsticks. And Curt already pointed out some concerns that he has with these steroid-like molecules and the phytosterols and we still have sensitization narration. So in ocular irritation, if it valuable. So there we're asking for literally every endpoint right now. If you can bring some set things in or just highlight the reports where you think we might want to bring information in, we can try and do it that way, but I mean this is insufficient and we have a list that I think everyone's agreed on correct?

**Dr. Liebler** - Yeah, I don't think we're going to be able to use the molecular weight as a get out of jail free card here. I mean that structure, the stratosphere, all Laurel glutamate on top of PDF 11. I mean, that's a that's roughly 750 molecular weight. It would be largely not absorbed it, but not completely, I think. It's always hard to predict, but. You know we it's not like a polymer where we can dismiss absorption completely.

**Dr. Belsito** - Right.

**Dr. Liebler** - So we just need to ask for the data and see what we get.

**Dr. Belsito** - OK. Any other comments? Allan. Paul, you've been very quiet.

**Dr. Rettie** - Well, it just seemed like we were going to ask for everything. So I was going to sit back and see what came in, but it was good to have this discussion because you're obviously looking to the next to the next iteration. The way you said, they're down about the molecular way. The other one, the octyldodecyl Laurel glutamate. We guess you're over 1000 with that one. That's probably one of the biggest, if not the biggest. So you're kind of a little and new man's land. Maybe some, you know, some dermal absorption for some and maybe less for others. Just based on the likelier weight. But it's a Gray area.

**Dr. Liebler** - Yep.

**Dr. Belsito** - Paul comments?

**Dr. Snyder** - I just felt that after your after you're opening insufficient data needs. I didn't think there was much to discuss at this point.

**Dr. Belsito** - OK.

**Dr. Liebler** - Right. Agree.

### Cohen's Team Meeting – June 16, 2022

[Due to technical difficulties, transcripts were not available for the Cohen team meeting.

### Full Panel – June 17, 2022

**Dr. Bergfeld -** OK. So moving on to the Phytosteryl glutamates Dr. Belsito.

**Dr. Belsito -** Yes, so. This includes three phytosterol glutamates. And they are mixed esters that each comprise laurel, would have tannic acid esterified with a mixture of phytosteryl *(inaudiable). Basically, this is the first time that we're looking at this, a document we did receive a significant amount of data but not overwhelming and I'm we were wondering to what extent we could bring in data from the fatty alcohols to Laurel, Lieutenaic acid, and the phytosterol reports that we previously had used or reviewed rather. But at this point we found this group to be insufficient for manufacturing impurities. 28-day dermal tox or other means of assessing absorption and depending upon absorption, other endpoints such as dark and genotoxicity may be needed. We need oral toxicity because it's used in lipsticks up to 25% sensitization and irritation, up to 25% for about a sterile opted vessel, glutamate. And we also need ocular irritation since it's used in high concentrations about the eye, *(inaudiable).

**Dr. Bergfeld -** And that's your motion.

**Dr. Belsito -** That's our motion.

**Dr. Bergfeld -** And you're going out for a what IDA?

**Dr. Belsito -** Yes.

**Dr. Bergfeld -** OK, Cohen?

**Dr. Cohen -** That's a second. We're perfectly harmonized.

**Dr. Bergfeld -** Very nice, good music, alright. I call the question, then all those opposed. Abstaining. Unanimously approved go out as an IDA with all those needs listed. Regina, do you need those listed again for you?

**Regina Tucker (CIR) -** No, I no, I have them. Thank you.

## DECEMBER 2022 PANEL MEETING – SECOND REVIEW/DRAFT TENTATIVE REPORT

### Belsito's Team Meeting – December 5, 2022

**DR. BELSITO:** So then we're going to move to Phytosteryl Glutamates. So this is a draft-tentative report. And after the June 2022 meeting, we issued an insufficient data announcement for the three ingredients, asking for method of manufacture, impurities data, 28-day dermal and, if positive, other tox endpoints, irritation and sensitization for Phytosteryl/Octyldodecyl Lauroyl Glutamate at maximum concentration of use, and ocular irritation

data if available. And we received a significant amount of data that was incorporated, both unpublished data on Phytosteryl/Octyldodecyl Lauroyl Glutamate, Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate.

And looking at that, I guess my first question is for the method of manufacture and impurities, was that small amount of data we got sufficient?

**DR. RETTIE:** Well, I noted that I felt we still more in chemical properties on method of manufacture because the information that we received was very minimal. For example, reference four, it's listed as anonymous information about MOM and impurities. It came in from PCPC and I kind of wondered if you could tell me how you view that anonymous material. Is it always fair game to accept it as it is?

**MS. EISENMANN:** So, companies don't want to give me method of manufacture. It's a very, very difficult thing to get. So, sometimes I can get a sentence out of them and that's all I can get. They just don't want to provide it because it's considered proprietary. It's something that I struggle with getting. You know, sometimes I have a better chance getting impurities information, but method of manufacture is very difficult.

**DR. RETTIE:** And in this case you didn't get much in the way of impurities beyond just arsenic and lead. I didn't really feel that I had very much information to go on for method of manufacture.

**DR. BELSITO:** So, Allan, you would say this is still insufficient for manufacturing and impurities?

**DR. RETTIE:** I'm, perhaps, over-channeling Dr. Liebler, but that's correct.

**DR. BELSITO:** Well, this is what we want you for. Paul, you would agree? I mean, that's why I raised the question.

**DR. RETTIE:** Yeah.

**DR. BELSITO:** To me it was like we didn't really get any information here.

**DR. RETTIE:** I agree with you.

**DR. SNYDER:** I mean, we did a thousand -- I mean, we did get a 28-day oral up to 1,000 and there was nothing toxicity-wise. So, I mean, unless there was something --

**DR. BELSITO:** But it's used 25 percent in lipsticks and we don't have anything greater than 28-days.

**DR. SNYDER:** Right. Yeah. I mean, it's a balance of -- you know, if there was a signal and what -- if there's something that we would -- there's certainly a deficiency but I'm not certain we're going to get any more, so.

**DR. BELSITO:** So, would the molecular weight help you to suggest that it's not absorbed? But since we don't have info on the manufacturer and impurities, we can't really assess what else might be in there that could cause problems?

**DR. RETTIE:** Yeah. I was more taken by the fact of the tox testing that came in that was so far below the maximum use concentration.

**DR. BELSITO:** Yeah.

**DR. RETTIE:** It's five percent if I was reading it correctly, but going up to 25 with use concentration.

**DR. BELSITO:** Right.

**DR. RETTIE:** Now there may be no signal, but whatever they used 1.5 percent, but what about 25 percent?

**DR. BELSITO:** I mean, in the animal studies, the oral short-term for 28-days, they went up to 1,000 mg/kg, which is pretty high. But it wasn't -- it was only for 28 days as opposed to a lipstick that you can put on a couple of times a day for years.

**DR. RETTIE:** I was unsure of why we included the gel permeation chromatography for the molecular weights. It seems like these could be calculated, right? And the gel permeation data are very specific: 388 to 1389. That's not typically the kinds of precision of numbers you get out of gel permeation chromatography experiments. I mean, if you calculate the molecular weights they're definitely going to be down in that range, so there is going to be some absorption.

**DR. BELSITO:** Yeah. So I thought it was still insufficient for manufacturing and impurities and for 28-day dermal tox since we're not getting a lot of impurities here. We do have sensitization and irritation at neat and ocular

irritation data at 1 percent. It's used at 12 percent around the eyes, but we only ask for ocular irritation available, we don't necessarily ask that it be done at concentration of use.

So at this point, I mean, since we're going insufficient, still, I would say manufacturing and impurities, 28-day dermal, if absorbed, DART, and ocular irritation if available at 12 percent.

**DR. RETTIE:** There was some new data in Wave 2 on ocular sensitivity.

**DR. BELSITO:** Yeah, it was at 1 percent.

**DR. RETTIE:** Restricted to Asian population.

**DR. BELSITO:** Yeah, I know.

**DR. RETTIE:** That's the only population they could get -- came from Asian. So, in that case, is photosensitivity something that you would want if ocular sensitivity pops up on a Wave 2 like this, which we don't believe was in the original report?

**DR. BELSITO:** Yeah, we always ask for ocular irritation, if available, if it's used around the eye.

**DR. RETTIE:** I was thinking photosensitivity, but maybe that's not what they're meaning here by ocular sensitivity.

**DR. BELSITO:** No.

**DR. RETTIE:** No. Okay. Thanks.

**DR. BELSITO:** Paul, comments?

**DR. SNYDER:** I mean, I was fine with that oral -- I mean, that oral for 1,000, but I'll go with the proposed insufficiency.

**MS. TUCKER:** Good morning. Was that manufacturing, impurities, 28-day dermal toxicity, DART and ocular irritation, if available, at 12 percent. Did I miss anything?

**DR. BELSITO:** Yes. So, manufacturing and impurities and a 28-day dermal are what are insufficient. If the 28-day dermal shows any evidence of absorption, then we would want other tox endpoints, specifically DART. And ocular irritation at maximum concentration of use, 12 percent if available.

**MS. TUCKER**: Thank you.

**DR. KLAASSEN:** Can you hear me?

**DR. BELSITO:** Yes, Curt.

**DR. KLAASSEN:** I would like to have maybe something put in the text about this group of compounds. So one of them is sitosterolemia. And actually, there are people that have toxicity due to this chemical because as you can see it's a steroid. And these are plant steroids. And animal steroids and plant steroids don't mix too well. And it turns out as a result of that, humans have a transporter in their intestine that prevents the absorption of these plant steroids.

And so, you know, there is a potential -- I don't think this is a significant problem with this dermal absorption, but within lipstick and going down into the intestine it could be. I think this should at least be mentioned, that we are aware that these plant steroids can cause diseases in some people.

I mean, we have evolved so the plant poisons wouldn't screw us up, but a few of us have mutations that plant steroids do screw us up. And this is one class of compound that this is well known.

**DR. RETTIE:** And those are mutations in the --

**DR. KLAASSEN:** And there's a lady down at Texas, Southwest that's worked all of this out very nicely in the last ten years. That's all.

**DR. BELSITO:** So Curt, that information is not here. So you're okay -- you want that data included but you're not requesting it as an insufficiency? Is that correct?

**DR. KLAASSEN:** That's correct. I want to make sure that people, when they read this report, realize that we know about this situation. But it doesn't need to affect the studies that you are requesting.

**DR. BELSITO:** Or require any additional data on the part of the panel?

**DR. KLAASSEN:** I don't think so.

**DR. RETTIE:** So just for my education, Curt, are those mutations a gut efflux transporter?

**DR. KLAASSEN:** Yes.

**DR. RETTIE:** In humans?

**DR. KLAASSEN:** Correct. And in laboratory animals, I think.

**DR. RETTIE:** Thank you.

**MS. FIUME:** Don? Can I ask for the purpose of the discussion, so the dermal irritation and sensitization studies are okay even though they're not at max concentration of use? Is there something we need to write in the discussion for that?

**DR. BELSITO:** Hold on. I have a note here that we have sensitization and irritation at neat levels.

**MS. FIUME:** So in the table, the mixture with the Phytosteryl/Octyldodecyl Lauroyl Glutamate is 5.9 -- basically 6 percent -- in formulation, and the formulation was tested neat. The face cream has 5 percent. The In Chemico, I believe, was neat, but the Human, the highest is 5 and 6 percent.

**DR. BELSITO:** Okay. So we have a negative DPRA, and then we have an HRIPT at 5 percent in 102 subjects. And the max concentration of use is what?

**DR. RETTIE:** Twenty-five.

**DR. BELSITO:** Twenty-five in a lipstick. That's right. Sorry, I read that wrong. I saw neat and I assumed it was tested neat.

**MS. FIUME:** We will clarify, in the summary, in text, that the mixture that tested neat is basically 6 percent of the ingredient, in the report, of the glutamate.

**DR. BELSITO:** Okay. And we need sensitization and irritation at maximum concentration of use.

**MS. FIUME:** So irritation, there is the one study --

**DR. BELSITO:** The irritation as neat.

**MS. FIUME:** Is neat.

**DR. BELSITO:** Yeah. And sensitization at concentration of use.

**MS. FIUME:** Okay. Thank you.

**DR. BELSITO:** And ocular irritation data at 12 percent if it's available.

**MS. FIUME:** Great. Thank you.

**MS. TUCKER**: So, we're adding the dermal?

**DR. BELSITO:** Yeah, sensitization at max use concentration, which is 25 percent, right?

**DR. KLAASSEN:** Yes.

**DR. BELSITO:** Anything else on this? So, to reiterate, Regina, it's insufficient for manufacture and impurities, 28-day dermal toxicity, if absorbed, DART data, sensitization at maximum use concentration, which we understand to be 25 percent. And ocular irritation data at 12 percent, if available. And then incorporate Curt's comments about --

**MS. TUCKER**: The sitosterolemia.

**DR. BELSITO:** Right.

**MS. TUCKER**: And that was that the plant steroids can cause disease in some people, something to that effect?

**DR. BELSITO:** With genetic defects. Curt, would you send that data on to the panel?

**DR. KLAASSEN:** Yes. Will do.

**DR. BELSITO:** Thank you. And that would be data, and that would also be part of the discussion. Okay. Anything else on the phytosteryl glutamates?

**Cohen's Team Meeting – December 5, 2022**

**DR. COHEN:** Next is Phytosteryl Glutamates. Okay. So Phytosteryl Glutamates, after reviewing the draft report in June 2022, we issued an IDA for the three ingredients with our data needs as method of manufacturing, impurities, 28-day dermal tox and, if positive, other tox endpoints. And we needed irritation and sensitization on lauroyl glutamate at maximum concentration of use, ocular irritation data if available. We have a method of manufacturing that was pretty cursory.

**DR. ROSS:** Yeah. It was not acceptable, yeah.

**DR. COHEN:** It was -- it's like how do you define a bridge? A bridge is something -- you get on a bridge. The impurities, I think, also were not that complete. We don't have an HRIPT at max use, only at 6 percent, but we do have a negative DPRA. Okay, so Susan, what do you think? It's a big report.

**DR. TILTON:** So, I noted that there were still insufficient data for the manufacturing and impurities. And -- trying to read my notes -- it's irritation and the sensitizing data were up to 5 percent.

**DR. ROSS:** 6 per- --

**DR. COHEN:** I think maybe it was like 5.99 or some- -- this is off the top of my head, but I have --

**DR. ROSS:** 5.999.

**DR. COHEN:** Yeah. It was like gold.

**DR. TILTON:** But it wasn't up to the max concentration.

**DR. COHEN:** No. No. Not even kind of close.

**DR. TILTON:** Ocular data was provided and was negative.

**DR. ROSS:** Not at max, though, right?

**DR. TILTON:** Make note of that.

**DR. ROSS:** I think the max was 12 percent.

**DR. COHEN:** Tom, what did you have down there? We'll come back around. Tom, are you on mute?

**DR. HELDRETH:** Yes, he is.

**DR. SLAGA:** There.

**DR. COHEN:** There you are.

**DR. SLAGA:** I'm on now.

**DR. COHEN:** What do you got for us on --

**DR. SLAGA:** Trying to keep the background noise down. What was your question?

**DR. COHEN:** Where do you stand on Phytosteryl Glutamates? What did your notes suggest? We felt we had insufficiencies --

**DR. SLAGA:** Same as you stated, yeah.

**DR. COHEN:** So, we still have insufficiencies on the manufacturing. What were our impressions of the impurities?

**DR. BERGFELD:** Arsenic was one.

**DR. COHEN:** Yes. And we only have impurities on one of them.

**DR. ROSS:** Yeah. That's not the one that's most commonly used.

**DR. COHEN:** Exactly.

**DR. BERGFELD:** I had a comment to make generally. This is a plant-derived chemical and basically a botanical plant-derived chemical.

**DR. ROSS:** Plant stearyl, right?

**DR. BERGFELD:** Yeah. So, we don't even mention the plant other than in some introductory comments here. Nor do we cover ourselves for the botanical boilerplates.

**DR. COHEN:** But these are refined chemicals from the plants, right?

**DR. BERGFELD:** Yeah, I know. But, plant-derived, not animal-derived. Plant. How do we deal with this?

**DR. HELDRETH:** So typically, when we have discrete chemicals, like we've laid out in the figure, we treat them for those constituents instead of --

**DR. BERGFELD:** So, I think in the discussion we should mention its plant derived and --

**DR. HELDRETH:** We can do that.

**DR. BERGFELD:** Yeah. Whatever you say about it. Whether you say it's a purified form of --

**DR. HELDRETH:** Right, it's refined, or it's been through processing.

**DR. BERGFELD:** Yeah. It has lead and arsenic as an impurity, so you think the boilerplate for heavy metal should be added?

**DR. HELDRETH:** Sure.

**DR. ROSS:** Yeah.

**DR. COHEN:** Mm-hmm.

**DR. BERGFELD:** You think pesticides or --

**DR. HELDRETH:** I mean, pesticides shouldn't be persistent in these products with the refinement and all the reactions that are done, but --

**DR. COHEN:** It'd be helpful to have the impurities just to be sure.

**DR. HELDRETH:** Right.

**DR. COHEN:** Right. Okay.

**DR. ROSS:** So, I had a list of what I felt was --

**DR. COHEN:** Please, because I'm compiling it now. I present this one.

**DR. ROSS:** So, just what you said about method of manufacturing/impurities that's being repeated. And was commonly used compound which I've abbreviated to P-O-L-G, dermal irritation and sensitization at max, as you've got only 6 percent as we've said. The ocular irritation potential at max. I think max is about 12 percent or 26 uses.

**DR. BERGFELD:** 26.5 or something, yeah.

**DR. ROSS:** Yeah, 26 uses.

**DR. COHEN:** Twelve percent by the eye, 25 by the lips. So, those are very sensitive areas. I mean, those are very high concentrations in these areas so we should have the data.

**DR. ROSS:** Yeah.

**DR. COHEN:** We need the data.

**DR. ROSS:** And I think all that -- that was actually on another compound, it was the Behneyl substituted compound. And it was done in a face cream at 1 percent in ocular cultures. I'm not quite sure how you put a face cream in an ocular culture, which is an aqueous reaction mix. But anyway --

**DR. COHEN:** I don't know how you'd do that.

**DR. ROSS:** -- I'm getting off point here, but you need that. And the other points, I've got notes here that says the Behneyl compound has no concentrations listed for 25 uses. So, we need to check what was the maximum concentration of that, just to make sure you've got irritation and sensitization in there.

**DR. COHEN:** Wait, for Behneyl. There are two Behneyls.

**DR. ROSS:** Oh, the -- yeah, sorry --

**DR. COHEN:** It's the lauroyl glutamate. Behneyl Lau- -- not the isostearyl one?

**DR. ROSS:** No. That's -- I'm coming to the isostearyl one.

**DR. COHEN:** Let me get this, if the Behneyl --

**DR. ROSS:** There was no concentrations, at least in my notes, and 25 uses. I got that right?

**DR. COHEN:** Yes, that's right.

**DR. ROSS:** So, I think we need some information on that just to check that we got the relevant dermal sensitization on that.

**DR. COHEN:** Concentration.

**DR. ROSS:** And then the last one with the isostearyl. We've only got one use but it's at a whopping 25.6 percent.

**DR. COHEN:** For the last one.

**DR. ROSS:** Yeah.

**DR. COHEN:** No concentration whereby the -- for eyes?

**DR. ROSS:** I don't think there was any concentrations reported at all. Yeah. No concentrations reported at all, David. That's the Phytosteryl, Behneyl, Octyldodecyl Lauroyl Glutamate.

**DR. COHEN:** Yeah. So, for these, if we're reading across, we're going to assume the highest concentration of the highest one in this case, right?

**DR. ROSS:** You know when I went through the transcript, I don't know about read across. But there didn't seem to be a great amount of enthusiasm for read across in last nights' transcript. That's my reading.

**DR. COHEN:** If we can't read across, should they be in the same report?

**DR. HELDRETH:** Yeah, we can certainly have -- I mean, read across is a fantastic rationale for grouping, but it's not the only one. You know, if for example anything you shared in common like manufacturing or impurities, or it's common to the group that you don't have to worry about a specific endpoint, it can be more efficient for the panel. You just have to be careful to make sure your conclusion is split between the different ingredients.

**DR. ROSS:** But you can make that point with respect to is there a read across or not and if not, we would need --

**DR. COHEN:** So, for the isostearyl, your comment was there's one use at high concentration?

**DR. ROSS:** It's 25.6 percent in a rouge. So, you would need some sort of dermal irritation/sensitization for that if you weren't reading across.

**DR. COHEN:** That's a bit of a bomb, right.

**DR. ROSS:** For one use, yeah.

**DR. COHEN:** Susan, what do you think?

**DR. TILTON:** So, I agree with the missing concentrations and test data at max concentrations. And then I also recall, from one of the prior discussions that was in the report about -- regarding concerns for read across for steroids in general, and due to differences in transport. So, it wasn't recommended previously. But I would agree with keeping them together. We just want to make sure we can evaluate them independently based on their usage.

**DR. ROSS:** And I think the read across comment was from Curt, I think.

**DR. TILTON:** Yeah.

**DR. COHEN:** Okay. I'm just compiling all these. So, if you can help me along with this. Insufficiencies remain on method of manufacturing, right? Because even what we have here is -- and we need it on all of them. Right?

**DR. ROSS:** Yeah.

**DR. COHEN:** We need impurities on all but the Octyldodecyl Lauroyl Glutamate, right?

**DR. ROSS:** No.

**DR. COHEN:** That's not right?

**DR. ROSS:** No. You have impurities on the Behneyl Octyldodecyl Lauroyl Glutamate.

**DR. COHEN:** Ah.

**DR. ROSS:** So, you need --

**DR. COHEN:** That one. All but --

**DR. ROSS:** All but that one.

**DR. COHEN:** Behnyl Octyldodecyl Lauroyl. Okay.

**DR. ROSS:** All but PBOLG.

**DR. COHEN:** That won't get me any closer to remembering what they are, though. We need HRIPT at max use. I wonder how tomorrow the negative DPRA will be utilized. I'm sure it'll be a very interesting conversation perhaps. We have some ocular data, but not at max use. And remind me for which one?

**DR. ROSS:** It's the most frequently used one. So it's the Phytosteryl Octyldodecyl Lauroyl Glutamate.

**DR. COHEN:** Wait --

**DR. ROSS:** That's what we need.

**DR. COHEN:** Yeah.

**DR. ROSS:** Because we're actually done with the Behneyl in the cultures at 1 percent.

**DR. BERGFELD:** I need a definition. What does MTT mean?

**DR. HELDRETH:** It's a test.

**DR. ROSS:** It's a tetrazolium test for mitochondria which actually measures, usually, cell viability.

**DR. HELDRETH:** Right. The MTT stands for --

**DR. BERGFELD:** So, if it doesn't affect MTT then it's okay?

**DR. ROSS:** Yeah. I think, I mean, that's basically how most people use it. Yeah.

**DR. COHEN:** And Behneyl Octyldodecyl Lauroyl Glutamate, we have no concentration reported?

**DR. ROSS:** Yep.

**DR. COHEN:** And the isostearyl glutamate, if we're not reading across we need HRIPT at max use, which is very high. Do we need anything else at that point if we're going to make -- because that's a blank row. It's a completely blank row. So, if we came back -- if next time we meet, we come back, they give us dermal sensitization, are we going to clear it if we have none of the other things and we're not reading across?

**DR. ROSS:** Well, that's a good question, yeah. I believe you can't.

**DR. COHEN:** You can?

**DR. ROSS:** I don't think you can clear it because you've got nothing else.

**DR. COHEN:** Do you have any advice on this one?

**DR. HELDRETH:** No, it seems like not all of your data needs were met. And it looks -- at least, it sounds like you're heading towards a tentative conclusion of insufficient data for all of the ingredients with those needs.

**DR. COHEN:** Yeah.

**DR. HELDRETH:** Now one thing I would want to point out, and maybe this is just splitting the infinitely small hair, but you said HRIPT, will you accept new approach methods like --

**DR. COHEN:** Yeah. So, let's do -- we don't have sensitization.

**DR. HELDRETH:** Okay.

**DR. COHEN:** Yeah. Yeah. Yeah.

**DR. HELDRETH:** Okay.

**DR. COHEN:** No, that's reasonable. Now, so the question is how DPRA will --

**DR. ROSS:** Look at my notes on that.

**DR. COHEN:** Because we've been in a few discussions where Don's group has felt that that was all they needed.

**DR. BERGFELD:** Right.

**DR. COHEN:** And we had that lecture about it. And I kind of go back and forth on this.

**DR. BERGFELD:** What does Tom think?

**DR. COHEN:** Tom, what do you think? We don't have sensitization at max use. We have it at about a quarter max use and --

**DR. SLAGA:** I thought we had sensitization. No?

**DR. COHEN:** At 6 percent.

**DR. BERGFELD:** Used at 25 plus. I think that's correct.

**DR. COHEN:** Yes. And we have a negative DPRA.

**DR. ROSS:** What concentration is that? That's a hundred millimolar.

**DR. COHEN:** Is that sort of a fixed type of -- is that a routinely fixed concentration? I would've expected that to be the case.

**DR. BJERKE:** Yes.

**DR. BERGFELD:** And what would that concentration translate to?

**DR. BJERKE:** Yeah, that's a good question. It depends on the context of use.

**DR. BERGFELD:** Are they active?

**DR. BJERKE:** But it is a standardized way and that's how it was validated.

**DR. COHEN:** It's looking how it co-ops with amino acids, right?

**DR. BJERKE:** That's right.

**DR. COHEN:** So, it either does or it doesn't, or it has to hit a certain threshold.

**DR. BJERKE:** That's right.

**DR. ROSS:** Point of discussion?

**DR. COHEN:** I think if I have to bet, I would suspect that the Belsito team might combine the lower concentration with a negative DPRA and clear it for that. And I'd like to hear that conversation. We're still missing a lot of other things here.

**DR. ROSS:** Yeah, still incomplete. Yeah.

**DR. COHEN:** Any thoughts on it?

**DR. BJERKE:** On the DPRA?

**DR. COHEN:** Yeah. In other words, we're at low sensitization -- low concentration/sensitization relative to the max use. And the max use are used in very sensitive areas. But then we have the negative DPRA, so the chances of it being a sensitizer are low.

**DR. BJERKE:** Yeah. Typically, for the new approach methodologies you don't rely on just one assay. It's not a complete replacement. So recall that we looked at the OECD497, which talked about the integrated testing strategies.

**DR. COHEN:** Yeah.

**DR. BJERKE:** So, you could -- in a situation if you were relying only on the new approach methodologies, you'd want to probably have three assays plus look at the structural alerts. Now, having said that, since you don't have that in its entirety to rely on that by itself, you could use a DPRA as part of weight of the evidence and then as a kind of an expert judgment ruling.

**DR. COHEN:** That's really helpful. Yeah. We're in a little bit of a gray zone here, because we have one in vitro assay, and we don't have a HRIPT at, say, 15 percent, it's at 25. Right? Where we can maybe fill the gap a little bit, this is much, much less. Okay.

**DR. BJERKE:** Do you have any in silico data looking at like a DEREK report or anything?

**DR. BERGFELD:** I didn't see it.

**DR. BJERKE:** That might be another approach where you can add to the weight of the evidence.

**DR. COHEN:** No, we listed both of those. Let's see, we have an EpiSkin going on. That was for non -- for irritation.

**DR. BERGFELD:** Yeah.

**DR. ROSS:** Yeah.

**DR. COHEN:** Okay.

**DR. BERGFELD:** You are presenting this?

**DR. COHEN:** I am. Yeah, that's okay. Yeah, no.

**DR. BERGFELD:** I'm just checking.

**DR. COHEN:** Yeah. No, it'll be fun when you say, do I have a second. That's when it gets fun.

**DR. BERGFELD:** But what do you have in your discussion right now? What are the points of controversy?

**DR. COHEN:** I'm just reopening it.

**DR. ROSS:** Just go through the asks.

**DR. COHEN:** Okay. So, we had the IDA before with the method of manufacturing, impurities, 28-day dermal tox. Further information if it was positive and then irritation and sensitization at max use. Ocular irritation if available. The insufficiencies remain on method of manufacturing for all of them. We need impurities on all but Behneyl-decyl-Lauroyl-Glutamate. We'd like sensitization data at max use or some other additional surrogate information. We have some ocular data, but not at max use for the most commonly used constituents. We have no concentration reported for the Behneyl Octyldodecyl-L-Glutamate. And for the isostearyl glutamate we have a quandary. Because if we're not reading across, we actually have nothing and so we'll need everything. We'll need method of manufacturing, impurities, dermal tox, oral tox, sensitization, irritation. So, the question will have to be the on chemists in the room.

**DR. BERGFELD:** So, are you clearing anything? It sounds like, no.

**DR. ROSS:** No.

**DR. BERGFELD:** It's all insufficient --

**DR. COHEN:** It's all inefficient.

**DR. BERGFELD:** -- and you're isolating them according to the ingredient?

**DR. ROSS:** Yeah.

**DR. BERGFELD:** Okay.

**DR. COHEN:** Good? Tom, okay with you?

**DR. SLAGA:** Yes. That sounds good to me.

**DR. COHEN:** Okay.

**DR. HELDRETH:** Regina, do you have everything you need for your discussion section?

**MS. TUCKER:** Yeah. So, for the discussion, mentioning that the ingredient is a plant-derived ingredient. Boilerplate for heavy metals and pesticides. The Behneyl has no concentration of use, so we want to check the max concentration. In isostearyl, one use at high concentration of 25.6 percent, dermal and irritation data is needed. If it was to be used as read across, method of manufacturing, limited on all. Sorry, impurities needed on all except for the --

**DR. SLAGA:** I can't hear the people in the back, so sorry.

**MS. TUCKER:** I'll try to yell it out a little bit louder for you. So, the method of manufacturing is limited on all. Impurities was also limited. Negative ocular on data but not at max use at 12 percent. Dermal and sensitization at max use, 25 percent. Does that sound like I covered everything?

**DR. ROSS:** That was with the Octyldodecyl Lauroyl Glutamate. So, the ocular and the dermal irritation and sensitization was for P-O-L-G -- POLG.

**DR. COHEN:** What was your correction again?

**DR. ROSS:** Just to specify that that was needed for the Octyldodecyl lauroyl Glutamate, which is the one with the most uses. For the dermal irritation and sensitization and the ocular. So, if we can get that at max use, I don't think you're too far from clearing that. So.

**DR. COHEN:** Well, we do need the sensitization.

**DR. ROSS:** Yes, we need that.

**DR. COHEN:** We need that sensitization data. It's of particular importance in this -- the concentrations are really high.

**DR. ROSS:** Yeah.

**DR. COHEN:** Okay. All right.

**Full Panel – December 6, 2022**

**DR. COHEN**: Phytosteryl Glutamates. After reviewing the draft report in June of 2022, an insufficient data announcement, on the three Phytosteryl Glutamates ingredients was issued with the following data needs: method of manufacturing, impurities, 28-day dermal tox and, if positive, further studies. Irritation and sensitization for Phytosteryl/Octyldodecyl Lauroyl Glutamate at maximum concentration of use, ocular irritation data if available.

Our motion is that there continues to be insufficiencies. Our needs are the following: manufacturer description for all. We thought the existing material was not adequate. Impurities for all but the Behenyl/Octyldodecyl Lauroyl Glutamate. We don't have sensitization at maximum use, only at five percent for Octyldodecyl Lauroyl, and we want that. We have a negative DART, but this is only one of the in vitro assays, and max uses in sensitive skin areas. We have some ocular data, but not at max use around the eyes for Phytosteryl/Octyldodecyl Lauroyl Glutamate, the most used item which we have at one percent with max use around the eyes at six percent.

**DR. BELSITO**: Twelve percent.

**DR. COHEN**: Twelve percent. Thank you. For the Behenyl/Octyldodecyl Lauroyl Glutamate, we have no concentration reported. And for Isostearyl Glutamate, we had a quandary if we're not reading across. And there was a discussion about not being able to read across. Not only would we need irritation and sensitization at max use, we probably need a full portfolio of data on that one. So, the motion is an IDA with the needs just described.

**DR. BELSITO**: We came to a similar conclusion. We did not discuss the inability to read across though. What was that based on?

**DR. COHEN**: It came up in the prior discussions.

**DR. ROSS:** Yes, it was a prior discussion on it and I think it was Curt who was discussing that, previously, whether or not it was appropriate to read across with these sterile components.

**DR. BERGFELD:** Curt, do you want to respond?

**DR. KLAASSEN**: Yes. I brought this up in our group yesterday as a general comment. We don't have any rules or regulations of when we could use read across and not read across. So, as you know, we have some compounds todays that are hair dyes that we used to read across, and now we can't read across. Scientifically, that's kind of disturbing.

But, what other compounds can't we read across? We've declared already that the prostaglandins, that we're going to be doing shortly, we can't read across. And so, I don't know what the reason for that is, although I agreed with it. Because they are work through receptors that have very distinctive chemical interactions to turn on various genes. And, so, I guess that's the reason for the prostaglandins. Now, using kind of a similar analogy, there are many, many different steroids in our body. And, all of these steroids are very similar in structure, if they have one hydroxyl here or a carbon in a little different place. And they act from everything from, in essence, corticosteroids to sex hormones to digoxin, digitoxin. And, as you know, all of those have very, very different properties and how can you read across?And so, I think chemicals that are in a class what I consider very active biologically, because they work their receptors, we got to be extremely careful in reading across. Now it just so happens with this

compound here, or a class of compounds, there is one chemical that's mentioned throughout this text, and it's sitosterol. Well, sitosterol is a plant steroid, and that's what we're talking about here is plant steroids.

It turns out that plant steroids and human steroids don't like each other. And so, in the intestines, we actually have a transporter, ABCG5/G8, I think, that transports sitosterol from the intestinal cells back into the gut, so we don't have that toxicity. Now there are a few people that have a mutation in that gene and they end up with having some serious problems.So, I guess, my whole bottom line is when do we use, and when is it wise to use read across and when is it not wise to read across. And, I definitely have problems reading across compounds that have structures similar to drugs and endogenous chemicals that work by activating receptors and increasing and decreasing various enzymes, protein structures, et cetera. So I'll stop at that.

**DR. BELSITO**: So, what you're saying, Curt, is you think each of these might interact with different receptors resulting in different biological effects?

**DR. KLAASSEN**: Yes, they might.

**DR. BELSITO**: So if they're not absorbed, though, is that an issue?

**DR. KLAASSEN**: Well, when we say they're not absorbed, that's somewhat nonscientific because there's nothing that's not absorbed to a small extent. And some of these compounds that act through receptors, act at a 10 to the -9th molar concentration. And, therefore, there's a concern here.

**DR. BELSITO**: But could we ask for a 28-day dermal on all three, which is what we've done, and clear them that way rather than "read across"?

**DR. KLAASSEN**: That would help.

**DR. COHEN**: What about the fact that there's 25 percent in lipsticks? It's very high concentrations around the eyes and the lips, which is what made us pause on this. I think if this had been low concentration on other body parts, we might not have held it.

**DR. BERGFELD:** Well, you've agreed that it should go out as an IDA?

**DR. BELSITO**: Insufficient, right.

**DR. BERGFELD:** Yeah.

**DR. BERGFELD:** Yeah. So, we're talking about a point of interest here.

**DR. COHEN**: Don, do you want to harmonize our insufficiencies, and then just look at the differences?

**DR. BELSITO**: There're pretty harmonized right now.

**DR. COHEN**: Okay.

**DR. BELSITO**: I think, perhaps -- I'm just looking at the document. You know, a search to see if any of these have been associated with -- I mean, your concern mainly is endocrine effects. Right Curt, endocrine disruption?

**DR. KLAASSEN**: Well, possibly, but not entirely that. The steroid molecule, and if you use the chemical structure of the steroid in a general way, they produce so many different effects. You know, digoxin and digitoxin are -- I don't know what the medicinal chemist think. They probably think I'm crazy when I say that they're somewhat structurally related to steroids, but to me they sure are. And, what they do is they affect the heart and the brain. So you don't know what's going to happen with these things until you test them. If they don't do anything, you're okay.

**DR. RETTIE:** Curt, can I make a comment?

**DR. KLAASSEN**: Please, I'd love you.

**DR. RETTIE:** I mean, I don't disagree with your comments around the universe of steroids, which are very, very potent bioactive materials, and they're endogenous. But I'm just looking at the chemical space around the hydrolysis products for the phytosteryls we have here. And, they are looking rather similar to me. We've got the same end product, if you will, at the level of the three alcohols that would be revealed by hydrolysis. We don't have any aromaticity in the a-ring. Sure they're decorated differently and I definitely don't know anything about their biological properties, even if it's relatively closely knitted, in my opinion.So, I kind of felt that that might help us here if we're only considering the three phytosteryls that I'm looking at on the screen here. Dave, you got any thoughts?

**DR. ROSS**: It's a difficult problem. Curt expressed his misgivings on small changes in these molecules having different effects of hydroxy group there, and, you know, hydroxy here can have all the difference. So even that they're relatively similar, even the cis-trans hydroxy group can causes changes particularly with steroid. So, I think, you just have to be careful. I'd just like to take you back to David's comment about the concentrations of these things that are being used. 25.6 percent or so in lipsticks and 12 percent or so in eye areas. So these are fairly high concentrations. So, I don't know, I'm not entirely comfortable with read across at this point.

**DR. RETTIE:** And I think I'd be in the same camp or much more in it if indeed there were hydroxylated moieties on these things.

**DR. ROSS:** Yeah.

**DR. RETTIE:** And, the ones I'm looking at here they don't have that. Even the stereo chemistry of methyl groups, the methyl groups are all exactly the same.

**DR. BELSITO**: Yeah. Well, let's see what we get if we get 28-day dermal, we can discuss this. But we agree with your data needs.

**DR. BERGFELD:** So, are we adding the 28-day dermal?

**DR. COHEN**: We'll add this.

**DR. BERGFELD:** And there's no read across at this point in time?

**DR. BELSITO**: Um-hmm. Bart?

**DR. HELDRETH**: So, since it sounds to me that the proposed insufficiencies are not outside the bounds of the original IDA, and therefore, since we're at the draft tentative stage, the Panel could choose instead to go forward with a tentative report with an insufficient data conclusion, instead of a second IDA.

**DR. BELSITO**: Sure.

**DR. COHEN**: What would happen next?

**DR. BELSITO**: It would be insufficient.

**DR. HELDRETH**: Yeah, it would go out as a tentative report for public comment with those insufficiencies listed in the discussion section explaining what was needed to come to a conclusion of safety. Typically, we reserve doing a second insufficient data announcement when we have new needs. But it seems like the needs we have are simply unmet needs from the original IDA.

**DR. COHEN**: Yeah, perfectly good idea.

**DR. BERGFELD:** So we're going to vote on a tentative final with some conclusion of safe and some split conclusion, here again, with needs. Is that correct?

**DR. BELSITO**: No, all unsafe.

**DR. COHEN**: No safety.

**DR. BERGFELD:** What's that?

**DR. BELSITO**: All insufficient.

**DR. COHEN**: All insufficient, no safety component.

**DR. BERGFELD:** All insufficient. Okay. And a tentative final.

**DR. BELSITO:** Um-hmm.

**DR. BERGFELD:** All right, we're going to call for the vote on that. All those in favor of that change, please indicate by hand. Thank you, unanimous. All right, well, we're going to move on then to the next ingredient, which is Octyldodecyl Stearoyl Stearate, Dr. Belsito.

# Safety Assessment of Phytosteryl Glutamates as Used in Cosmetics

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The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Thomas J. Slaga, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. Previous Panel members involved in this assessment: Daniel C. Liebler, Ph.D., and Ronald C. Shank, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D., and the Senior Director is Monice Fiume. This report was prepared by Wilbur Johnson, Jr., M.S., former Senior Scientific Analyst/Writer, and Regina Tucker, M.S., Scientific Analyst/Writer, CIR.


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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 |
| 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 relevant data to determine the safety of these ingredients. Industry should continue to use good manufacturing practices to limit impurities, such as heavy metals, in cosmetic formulations. The Panel concluded that the available data are insufficient to make a determination of safety for these phytosteryl glutamate ingredients under the intended conditions of use in cosmetic formulations.


## 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.

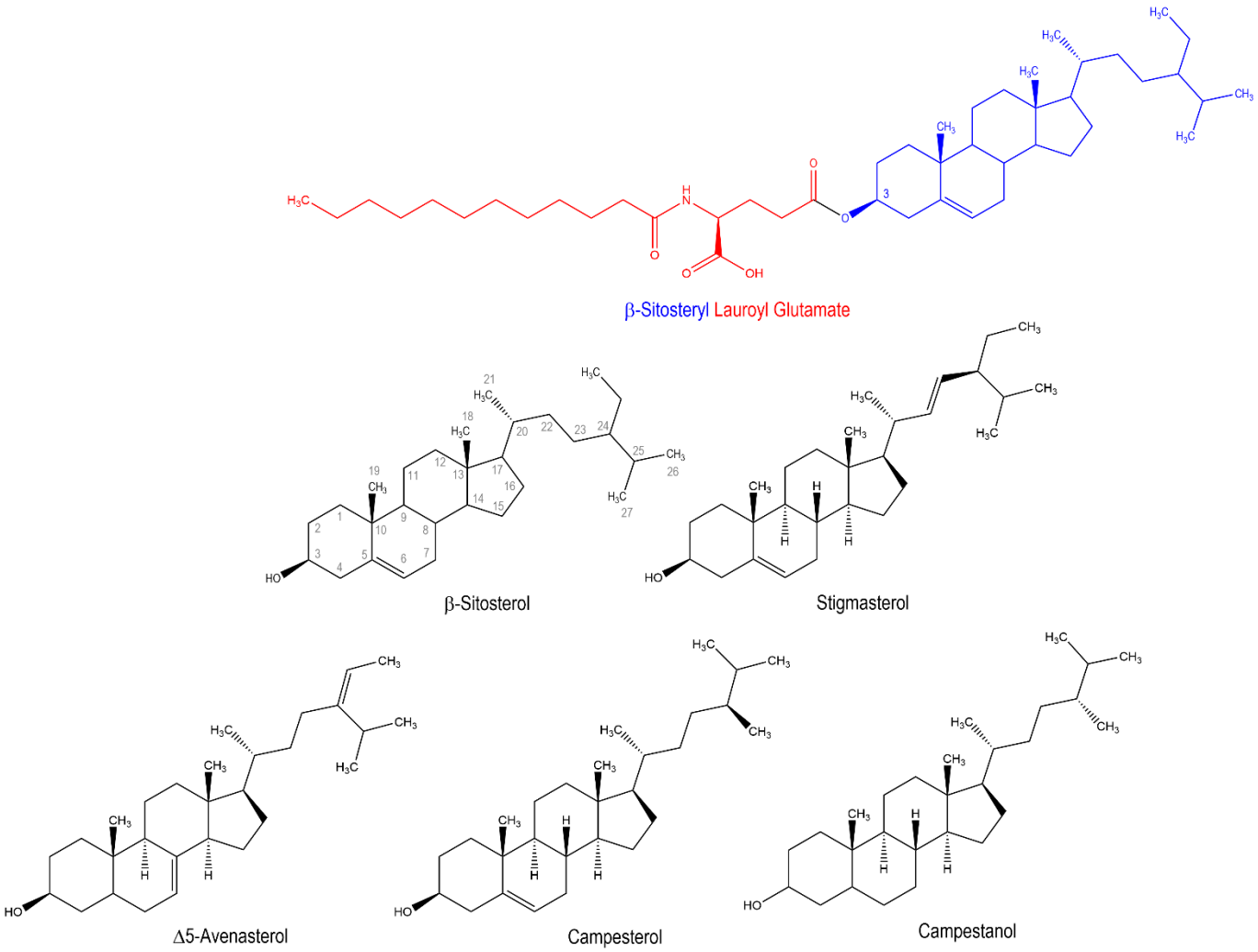


**Figure 1**. Phytosterols and phytosteryl connectivity

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)).

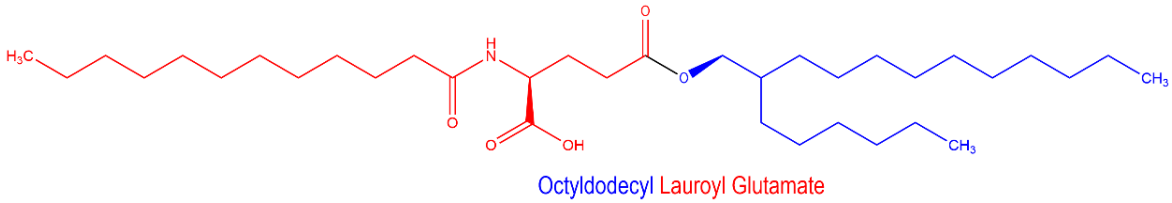


**Figure 2.** Octyldodecyl Lauroyl Glutamate

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 Food and Drug Administration (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.

In another short-term oral toxicity study, Phytosteryl/Octyldodecyl Lauroyl Glutamate was administered by gavage to 5 male and 5 female Wistar rats at dose levels of 0, 50, 200, and 1000 mg/kg/d for 28 d.[13] The no-observed-effect level (NOEL) was 1000 mg/kg/d.

### 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 was negative in its potential to induce structural chromosome aberrations in a Chinese hamster lung cell line (CHL/IU) at concentrations between 0.625 - 5.0 µg/ml.[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. In an in vitro cell viability assay using EpiSkin™ reconstituted human epidermis, a product containing 1% Phytosteryl/Octyldodecyl Lauroyl Glutamate was deemed to be non-irritating.[18] The skin irritation potential of all three phytosteryl glutamates (each tested at 100%) was evaluated on New Zealand white rabbits (groups ranging from 3 - 6 animals) conducted under a semi-occlusive or occlusive patch; all were found to be non-irritating.[13] A 14-d open-application cumulative 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 (undiluted) tested on 31 human subjects was deemed to be a non-irrtant.[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 on 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 study with a face cream 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 was prepared as a 100 mM stock solution and tested for cysteine and lysine depletion, both peptides showed minimal reactivity.[21] Three separate guinea pig maximization tests on 15 female guinea pigs were negative when performed on the three phytosteryl glutamates (concentrations ranging up to 100%).[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 a sensitizer.[22] In another HRIPT, a mixture containing 5.99% 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 test the ocular irritancy 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/d in a short term-toxicity study on Phytosteryl/Octyldodecyl Lauroyl Glutamate.

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.

An in-vitro cell viability assay of 1% Phytosteryl/Octyldodecyl Lauroyl Glutamate using EpiSkin™ reconstituted human epidermis was predicted to be non-irritating. An in-vitro human skin model test was non-corrosive when performed on Phytosteryl/Behenyl/Octyldodecyl Isostearyl Lauroyl Glutamate (concentration not stated). A skin irritation assay performed on the same ingredient (concentration not stated), using EpiDerm™, was not classified. All three phytosteryl glutamates (each tested at 100%) were non-irritating in a study on New Zealand white rabbits conducted under semi-occlusive or occlusive patch. A 14-d open-application cumulative skin irritation study on 10 female guinea pigs with Phytosteryl/Behenyl Octyldodecyl Lauroyl Glutamate and Phytosteryl Octyldodecyl Lauroyl Glutamate at maximum concentrations of 100% yielded no skin reactions.

In a 24-h occlusive patch test, a Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate cream (undiluted) tested on 31 human subjects was deemed to be a non-irritant Two separate 24-h patch tests of Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate and Phytosteryl/Octyldodecyl Lauroyl Glutamate (both at 100%) were negative for irritation on 45 human subjects. A face cream containing 1% Phytosteryl/Behenyl/Octyldodecyl Lauroyl Glutamate was determined to be non-irritating in 25 subjects. A 7-d semi-occlusive cumulative irritation patch study with a face cream containing 1.5% Phytosteryl/Octyldodecyl Lauroyl Glutamate resulted in no irritation in 38 subjects.

In a DPRA of Phytosteryl/Octyldodecyl Lauroyl Glutamate, both peptides showed minimal reactivity. Three separate guinea pig maximization tests on 15 female guinea pigs were negative when performed on the three phytosteryl glutamates (concentrations ranging up to 100%). In an HRIPT, a face cream containing 5% Phytosteryl/ Behenyl/Octyldodecyl Lauroyl Glutamate was not a sensitizer when tested on 102 subjects. In another HRIPT, a mixture containing 5.99% Phytosteryl/Octyldodecyl Lauroyl Glutamate (219 subjects) was not an irritant or a sensitizer.

A tissue equivalent assay was conducted to test the ocular irritancy 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. 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

This assessment reviews the safety of 3 phytosteryl glutamates. The Panel concluded that the available data are insufficient for determining the safety of these ingredients under the intended conditions of use in cosmetics. The Panel noted an overall lack of relevant safety data and determined that the data needs from the Insufficient Data Announcement from the June 2022 Panel meeting remain unmet. In order to come to a conclusion of safety for these cosmetic ingredients, the following additional data are needed:

- method of manufacturing
- impurities
- 28-day-dermal toxicity
    - if positive, other toxicological endpoints (e.g., developmental, and reproductive toxicity, genotoxicity, carcinogenicity, etc.) may be needed
- sensitization and irritation data for Phytosteryl/Octyldodecyl Lauroyl Glutamate at maximum concentration of use
- ocular irritation, if available

The Panel discussed the plant steroid sitosterol and the possible biological effects it causes 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 current good manufacturing practices (cGMPs) to limit impurities in cosmetic formulations.

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 available data are insufficient to make a determination of safety for the following 3 phytosteryl glutamates under the intended conditions of use in cosmetic formulations:

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<br>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<br>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<br>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)<br>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 | [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™) | 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 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, applied undiluted. | 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<br>8 males<br>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 study 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)<br>Epidermal induction 100%<br>Challenge 100% | 15 Female Dunkin-Hartley Albino guinea pigs<br>10 test group<br>5 control | A maximization test was performed. Test sites were observed 24 and 48 h after removal of the application patch. | Negative | 13 |
| Phytosteryl/Behenyl/ Octyldodecyl/Isostearyl Lauroyl Glutamate | Intradermal induction 10% (in liquid paraffin)<br>Epidermal induction 100%<br>Challenge 50, 100% (PEG 300, vehicle) | 15 Female Hartley Albino guinea pigs<br>10 test group<br>5 control | OECD 406. A maximization test was performed. Test sites were observed 24 and 48 h after removal of the application patch. | Negative | 13 |
| Phytosteryl/Octyldodecyl Lauroyl Glutamate | Intradermal induction 5% (in PEG 400)<br>Epidermal induction 100%<br>Challenge 10% (in PEG 400) | 15 female Himalayan spotted guinea pigs<br>10 test group<br>5 control | A maximization test was performed. Test sites were observed 24 and 48 h after removal of the application 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.<br>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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Personal Care Products Council
Committed to Safety, Quality & Innovation

**Memorandum**

**TO:** Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

**FROM:** Carol Eisenmann, Ph.D.
Personal Care Products Council

**DATE:** February 13, 2023

**SUBJECT:** Phytosteryl Glutamates

Anonymous. 2023. The manufacturing methods: Phytosteryl glutamates.

Anonymous. 2023. Safety Data Summary: Phytosteryl glutamates.

# The Manufacturing methods

### PHYTOSTERYL/ OCTYLDODECYL LAUROYL GLUTAMATE

Lauroyl glutamic acid
↓
↓←Phytosterol, Octyldodecanol
↓
<Esterification> (Acid catalyst)
↓
↓
↓
<Purification> (Alkaline aqueous solution)
↓
↓← Tocopherol
↓
Final product

### PHYTOSTERYL/BEHENYL/ OCTYLDODECYL LAUROYL GLUTAMATE

Lauroyl glutamic acid
↓
↓←Phytosterol, Behenyl alcohol, Octyldodecanol
↓
<Esterification> (Acid catalyst)
↓
↓
↓
<Purification> (Alkaline aqueous solution)
↓
↓← Tocopherol
↓
Final product

### PHYTOSTERYL/BEHENYL/ OCTYLDODECYL/ISOSTEARYL LAUROYL GLUTAMATE

Lauroyl glutamic acid
↓
↓←Phytosterol, Behenyl alcohol, Octyldodecanol, Isostearyl alcohol
↓
<Esterification> (Acid catalyst)
↓
↓
↓
<Purification> (Alkaline aqueous solution)
↓
↓← Tocopherol
↓
Final product

| | PHYTOSTERYL/ OCTYLDODECYL LAUROYL GLUTAMATE | PHYTOSTERYL/BEHENYL/ OCTYLDODECYL LAUROYL GLUTAMATE | PHYTOSTERYL/BEHENYL/ OCTYLDODECYL/ ISOSTEARYL LAUROYL GLUTAMATE |
|---|---|---|---|
| LAURIC ACID | ✓ | ✓ | ✓ |
| L-GLUTAMIC ACID | ✓ | ✓ | ✓ |
| PHYTOSTERYL ALCOHOL | ✓ | ✓ | ✓ |
| BEHENYL ALCOHOL | | ✓ | ✓ |
| 2-OCTYLDODECANOL | ✓ | ✓ | ✓ |
| ISOSTEARYL ALCOHOL | | | ✓ |

- Residue of starting materials, such as phytosterol, behenyl alcohol, octyldodecanol and isostearyl alcohol and lauroyl glutamic acid (cosmetic ingredients), may remain in the final products.
- Heavy metals: Heavy metals (as Pb); less than 20ppm
- Arsenic: Arsenic (as $As_2O_3$); less than 2ppm
- Acid catalyst: Esterification is performed using an acid catalyst, and lauroyl glutamic acid and acid catalyst are removed with alkaline aqueous solution. Water is then added for oil separation, to remove inorganic salts and lauroyl glutamic acid.
- Pesticide: The possibility of pesticide contamination in the above flows is extremely low.

# Summary Table

| | PHYTOSTERYL/OCTYLDODECYL LAUROYL GLUTAMATE | PHYTOSTERYL/BEHENYL/OCTYLDODECYL LAUROYL GLUTAMATE | PHYTOSTERYL/BEHENYL/OCTYLDODECYL/ ISOSTEARYL LAUROYL GLUTAMATE |
|---|---|---|---|
| **Acute oral toxicity** | | | |
| Study period | 1999/6/22 | 1997/12/5 | 2009/7/20 |
| Test method | Directive 92/69 EEC, B.1 tris., OECD Guidelines No. 423 | Limit test | OECD Guidelines No. 423 |
| Test system | Male and Female Rats (HanBrl: Wist) | Male and Female ICR Mice | Wister Rat (Crl:WI) |
| Number of animals | 3 Males and 3 Females | 5 Males and 5 Females / Group | 3 females×2 group |
| Dose levels | 2000 mg/kg | 1.0 g/kg , 2.0 g/kg and vehicle (control) | Corn oil |
| Vehicle | PEG 300 | Olive oil | 14 days after treatment |
| Observation period | 14 days after treatment | 14 days after treatment | 2000 mg/kg |
| Result and conclusion | $LD_{50}$ > 2000 mg/kg (No death occurred) | $LD_{50}$ > 2.0g/kg (No death occurred in all groups) | $LD_{50}$(rat) > 2000mg/kg (No death occurred in all groups)<br>*This Acute oral toxicity test was not conducted for cosmetic purpose but for industrial purpose. |
| **Acute dermal toxicity** | | | |
| Study period | 1999/6/21 | | |
| Test method | Directive 92/69/EEC, B.3., OECD Guidelines No. 402 | | |
| Test system | Male and Female Rats (HanBrl: Wist) | | |
| Number of animals | 5 Males and 5 Females / Group | | |
| Dose levels (Dose volume) | 2000 mg/kg (2.16mL/kg) | | |
| Observation period | 14 days after treatment | | |
| Result and conclusion | $LD_{50}$ > 2000 mg/kg (No death occurred) | | |
| **28-Day oral toxicity (Gavage)** | | | |
| Study period | 1999/7/23 | | |
| Test system | Male and Female Rats (HanBrl: Wist) | | |
| Number of animals | 5 Males and 5 Females / Group | | |
| Daily Dose levels | 0, 50, 200, 1000 mg/kg/day | | |
| Vehicle | PEG 300 | | |
| Dose volume | 5 mL/kg body weight | | |
| Frequency of administration | Daily | | |
| Duration of treatment | 28 days | | |
| Result and conclusion | NOEL (no-observed-effect-level); 1000 mg/kg/day | | |
| **Primary skin irritation** | | | |
| Study period | 1999/6/23 | 1997/10/22 | 2004/9/8 |
| Test method | Directive 92/69 EEC, B.4., OECD Guideline 404 (4-Hour Semi-Occlusive application) | Draize test (24hrs occlusive patch) | OECD Guidelines No. 404 (4-Hour Occlusive Application) |
| Test system | Male and Female New Zealand White rabbits | Male New Zealand White rabbits | New Zealand White Rabbit |
| Number of animals | 1 Male and 2 Females | 6 Males | 3 males |
| Concentration of test article | 100% | 100% | 100.0% |
| Result and conclusion | "Not irritating" to rabbit skin<br>*Irritation level was classified according to "EEC Commission Directive 93/21/EEC of April 27, 1993" | Non irritant | "Not irritating" to the rabbit skin<br>* Irritation level was classified according to"EEC Commission Directive 2001/59/EC" . |
| | Observation period after treatment: 1hr / 24hr / 48hr / 72hr<br>Mean score: 0.00 / 0.00 / 0.00 / 0.00 | Observation period after treatment: 24hr / 48hr / 72hr / 1week<br>Mean score: 0.0 / 0.0 / 0.0 / 0.0 | Observation period after treatment: 1hr / 24hr / 48hr / 72hr<br>Mean score: 0.0 / 0.0 / 0.0 / 0.0 |
| ***in vitro* Skin corrosion:Human skin model test (MTT assay)** | | | |
| Study period | | | 2009/2/5 |
| Test method | | | OECD Guideline No. 431 |
| Result and conclusion | | | Non-corrosive |
| ***in vitro* Skin irritation: Epiderm™** | | | |
| Study period | | | 2011/9/6 |
| Test method | | | OECD Guideline No. 439 |
| Result and conclusion | | | Not classified |
| **Cumulative skin irritation in guinea pigs** | | | |
| Study period | 1997/12/18 | 1997/12/18 | |
| Test method | Open application (14days) | Open application (14days) | |
| Test system | Female Dunkin-Hartley albino guinea-pigs | Female Dunkin-Hartley Albino guinea-pigs | |
| Number of animals | 10 Females | 10 Females | |
| Concentration of test article | 0, 10, 30 and 100%(W/W) (Vehicle : vaseline) | 10, 30 and 100%(W/W)(A.I.) (Vehicle: Vaseline) | |
| Application method | Open application | Open application | |
| Application period | 14 days | 14 days | |
| Result and conclusion | No skin reaction was observed in all concentrations | Non irritating (No skin reaction was observed at any concentrations) | |

# Summary Table

## Skin sensitization

| | PHYTOSTERYL/OCTYLDODECYL LAUROYL GLUTAMATE | PHYTOSTERYL/BEHENYL/OCTYLDODECYL LAUROYL GLUTAMATE | PHYTOSTERYL/BEHENYL/OCTYLDODECYL/ ISOSTEARYL LAUROYL GLUTAMATE |
|---|---|---|---|
| Study period | 1999/6/15 | 1997/11/14 | 2008/7/24 |
| Test method | Maximization test (Magnusson and Kligman method) | Maximization test (Magnusson and Kligman method) | OECD Guidelines No. 406 |
| Test system | Female guinea-pigs (Ibm: GOHI, synonym: Himalayan spotted) | Female Dunkin-Hartley albino guinea-pigs | Female Albino guinea pigs (Hartley) |
| Number of animals | 15 (10 for test group, 5 for control group) | 15 (10 for test group, 5 for control group) | 15 (10 for the test group, 5 for the control group) |
| Concentration of test article | Intradermal induction : 5% (Vehicle: PEG 400 )<br>Epidermal induction : 100%<br>Challenge : 10% (Vehicle: PEG 400 ) | Intradermal induction: 25%(W/W)(A.I.) (Vehicle: Olive oil)<br>Epidermal induction: 100%<br>Challenge: 100% | Intradermal induction: 10%(W/W) (A.I.) (Vehicle: Liquid paraffin)<br>Epidermal induction: 100%(W/W) (A.I.)<br>Epidermal challenge: 100% and 50%(W/W) (A.I.) (Vehicle: PEG 300) |
| Observation period | 24 and 48 hours after removal of the application patch | 24 and 48 hours after removal of the application patch | 24 and 48 hours after removal of the application patch |
| Result and conclusion | Negative | Negative | Negative |

## Primary eye irritation

| | PHYTOSTERYL/OCTYLDODECYL LAUROYL GLUTAMATE | PHYTOSTERYL/BEHENYL/OCTYLDODECYL LAUROYL GLUTAMATE | PHYTOSTERYL/BEHENYL/OCTYLDODECYL/ ISOSTEARYL LAUROYL GLUTAMATE |
|---|---|---|---|
| Study period | 1999/6/23 | 1997/11/26 | 2004/12/8 |
| Test method | Directive 92/69 EEC, B.5., OECD Guideline 405 | Draize test | OECD Guidelines No.405 |
| Test system | Male and Female New Zealand White rabbits | Male New Zealand White rabbits | New Zealand White Rabbit |
| Number of animals | 1 Male and 2 Females | 6 Males | 3 males |
| Concentration of test article | 100% | 10%(W/W)(A.I.) (Vehicle: Olive oil) | 100.0% |
| Result and conclusion | "Not irritating" to rabbit eye | Non-irritating | "Not irritating" to the rabbit eye |

*Irritation level was classified according to "EEC Commission Directive 93/21/EEC of April 27, 1993"

| Observation period after treatment | 1hr | 24hr | 48hr | 72hr |
|---|---|---|---|---|
| Mean score | 1.00 | 0.00 | 0.00 | 0.00 |

* Irritation level was classified according to "Kay and Calandra method"

| treatment | 24hr | 48hr | 72hr | 96hr | 1week |
|---|---|---|---|---|---|
| Mean score | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

* Irritation level was classified according to "EEC Commission Directive 2001/59/EC" .

| Observation period after treatment | 1hr | 24hr | 48hr | 72hr |
|---|---|---|---|---|
| Cornea | 0.0 | 0.0 | 0.0 | 0.0 |
| Iris | 0.0 | 0.0 | 0.0 | 0.0 |
| Conjunctiva Redness | 1.0 | 1.0 | 0.0 | 0.0 |
| Conjunctiva Chemosis | 0.3 | 0.0 | 0.0 | 0.0 |

## *in vitro* Ocular irritation :EpiOcular™

| | PHYTOSTERYL/OCTYLDODECYL LAUROYL GLUTAMATE | PHYTOSTERYL/BEHENYL/OCTYLDODECYL LAUROYL GLUTAMATE | PHYTOSTERYL/BEHENYL/OCTYLDODECYL/ ISOSTEARYL LAUROYL GLUTAMATE |
|---|---|---|---|
| Study period | | | 2009/2/5 |
| Test method | | | EpiOcular™ |
| Result and conclusion | | | non/minimal irritant. |

## Bacterial reverse mutation test

| | PHYTOSTERYL/OCTYLDODECYL LAUROYL GLUTAMATE | PHYTOSTERYL/BEHENYL/OCTYLDODECYL LAUROYL GLUTAMATE | PHYTOSTERYL/BEHENYL/OCTYLDODECYL/ ISOSTEARYL LAUROYL GLUTAMATE |
|---|---|---|---|
| Study period | 1999/5/14 | 1997/12/9 | 2005/3/5 |
| Test method | "Plate incorporation test" and "Pre-incubation test" | Pre-incubation method | OECD Guidelines No.471 |
| Test system | *S. typhimurium* TA98, TA100, TA1535, TA1537<br>*E. coli* WP2 *uvrA* | *S. typhimurium* TA98, TA100, TA1535, TA1537<br>*E. coli* WP2 *uvrA* | *S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538<br>*E. coli* WP2 *uvrA* |
| Vehicle | Acetone | Acetone | DMSO |
| Concentration of test article | Plate incorporation test 5000, 2500, 1000, 333, 100, 33μg/plate (with and without S9-mix)<br>Pre-incubation test 5000, 2500, 1000, 333, 100, 33μg/plate (with and without S9-mix) | | Plate incorporation test 5000, 3160, 1000, 316, 100 μg/plate (with and without S9-mix)<br>Pre-incubation test 5000, 3160, 1000, 316, 100 μg/plate (with and without S9-mix) |

Toxic effect concentration in Pre-incubation test (μg/plate)

| Strain | TA1535 | TA1537 | TA98 | TA100 | WP2 *uvrA* |
|---|---|---|---|---|---|
| S9-mix(-) | 1000-5000 | 33-5000 | 5000 | 1000 | / |
| S9-mix(+) | / | 1000 | / | / | / |

/ : No relevant toxic effects observed

| Strain | S9-mix(-) | consentration(μg/plate) | S9-mix(+) | consentration(μg/plate) |
|---|---|---|---|---|
| TA1535 | 1250 p, 625 p, 313, 156, 78 | | 1250 p, 625, 313, 156, 78 | |
| TA1537 | 313 t, 156, 78, 39, 20, 10 | | 1250 p, 625, 313, 156, 78 | |
| TA98 | 313 t, 156, 78, 39, 20, 10 | | 1250 p, 625, 313, 156, 78 | |
| TA100 | 1250 p, 625 p, 313, 156, 78 | | 1250 p, 625, 313, 156, 78 | |
| WP2 *uvrA* | 1250 p, 625 p, 313, 156, 78 | | 1250 p, 625, 313, 156, 78 | |

p: precipitation, t: toxic effect

| | PHYTOSTERYL/OCTYLDODECYL LAUROYL GLUTAMATE | PHYTOSTERYL/BEHENYL/OCTYLDODECYL LAUROYL GLUTAMATE | PHYTOSTERYL/BEHENYL/OCTYLDODECYL/ ISOSTEARYL LAUROYL GLUTAMATE |
|---|---|---|---|
| Result and conclusion | Negative | Negative | Negative (Non-mutagenic) |

## *in vitro* Mammalian chromosome aberration test

| | PHYTOSTERYL/OCTYLDODECYL LAUROYL GLUTAMATE | PHYTOSTERYL/BEHENYL/OCTYLDODECYL LAUROYL GLUTAMATE | PHYTOSTERYL/BEHENYL/OCTYLDODECYL/ ISOSTEARYL LAUROYL GLUTAMATE |
|---|---|---|---|
| Study period | 1999/9/15 | 1998/10/2 | |
| Test system | Cell line: V79 (Chinese hamster cell line) | Cell line: CHL/IU | |
| Vehicle | Acetone | Acetone | |

| Exposure | Recovery | preparation interval | S9-mix | Concentration (μg/mL) |
|---|---|---|---|---|
| 4hr | 14hr | 18hr | S9-mix(-) | 2500.0, 1250.0, 625.0, 156.3, 78.1 |
| 4hr | 14hr | 18hr | S9-mix(+) | 300.0 p, 75.0, 37.5, 18.8, 9.4 |
| 4hr | 24hr | 28hr | S9-mix(+) | 2500.0, 312.5, 156.3, 78.1 |
| 18hr | - | 18hr | S9-mix(-) | 2500.0, 1250.0, 625.0, 156.3, 78.1 |
| 28hr | - | 28hr | S9-mix(-) | 2500.0, 1250.0 |

p: precipitation

| Exposure | Recovery | preparation interval | S9-mix | Concentration (μg/mL) |
|---|---|---|---|---|
| 6hr | 18hr | 24hr | S9-mix(-) | 5.0 p, 2.5 p, 1.25 p, 0.625 p |
| 6hr | 18hr | 24hr | S9-mix(+) | 5.0 p, 2.5 p, 1.25 p, 0.625 p |
| 24hr | - | 24hr | S9-mix(-) | 5.0 p, 2.5 p, 1.25 p, 0.625 p |
| 48hr | - | 48hr | S9-mix(-) | 5.0 p, 2.5 p, 1.25 p, 0.625 p |

p: precipitation

| | PHYTOSTERYL/OCTYLDODECYL LAUROYL GLUTAMATE | PHYTOSTERYL/BEHENYL/OCTYLDODECYL LAUROYL GLUTAMATE | PHYTOSTERYL/BEHENYL/OCTYLDODECYL/ ISOSTEARYL LAUROYL GLUTAMATE |
|---|---|---|---|
| Result and conclusion | Negative | Negative | |

# Summary Table

| | PHYTOSTERYL/OCTYLDODECYL LAUROYL GLUTAMATE | PHYTOSTERYL/BEHENYL/OCTYLDODECYL LAUROYL GLUTAMATE | PHYTOSTERYL/BEHENYL/OCTYLDODECYL/ ISOSTEARYL LAUROYL GLUTAMATE |
|---|---|---|---|
| *in vitro* Mammalian Cell Gene Mutation Test in Mouse Lymphoma | | | |
| Study period | 2012/3/6 | | |
| Test system | Mouse lymphoma L5178Y/TK$^{+/-}$ cells | | |
| Concentration of test article | Exposure / S9-mix / Concentration (μg/mL)<br>4hr / S9-mix(-) / 5000, 2700, 900, 300, 100, 10<br>4hr / S9-mix(+) / 5000, 2700, 900, 300, 100, 10 | | |
| Result and conclusion | Negative | | |
| Humans patch test | | | |
| study period | 1997/12/10 | 1997/12/10 | |
| Test method | Occlusive application (24-hours, crooked side of upper arm) | Occlusive application (24-hours, crooked side of upper arm) | |
| No. of volunteers | 45 (Japanese: 8 Males, 37 Females) | 45 (Japanese: 8 Males, 37 Females) | |
| Concentration of test article | 100%(W/W)(A.I.) | 100% | |
| Result and conclusion | Negative (Non irritating) | Negative (Non irritating) | |