Data Supplement

4-Chloro-2-Aminophenol

Fatty Amphocarboxylates

Inositol

Paeonia suffruticosa

p-Phenylenediamine

Prostaglandin Analogues

1,2,4-Trihydroxybenzene

Yeast-derived ingredients

EXPERT PANEL MEETING
June 3-4, 2024

Commitment & Credibility since 1976

Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Christina L. Burnett, MSES, Senior Scientific Analyst/Writer, CIR
Date: May 24, 2024
Subject: Wave 2 - Amended Safety Assessment of 4-Chloro-2-Aminophenol as Used in Cosmetics

Please find attached the comments provided by the Personal Care Products Council on the Draft Amended Report on 4-Chloro-2-Aminophenol (*PCPCcomments_4-Chloro-2-Aminophenol_Wave2_062024*).

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: May 21, 2024

SUBJECT: Draft Report: Amended Safety Assessment of 4-Chloro-2-Aminophenol as Used in Cosmetics (draft prepared for the June 2024 meeting)

The Personal Care Products Council respectfully submits the following comments on the draft report, Amended Safety Assessment of 4-Chloro-2-Aminophenol as Used in Cosmetics.

Cosmetic Use; Summary – The Proposition 65 listing as a carcinogen does not belong in the Cosmetic Use section. It should be included in the Carcinogenicity section with the IARC classification. Generally, California lists chemicals on Proposition 65 after another authoritative body, such as IARC has listed it.

Subchronic – Please correct: “near the end of the end of the treatment period” (delete one “the end of”)

Genotoxicity, old report summary – Was 4-Chloro-2-Aminophenol weakly mutagenic with or without metabolic activation (or under both conditions)?

Carcinogenicity – It would be helpful to note what year the IARC 2B determination was made.

Cosmetic Ingredient Review

Commitment & Credibility since 1976

Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Priya Cherian, MS, Senior Scientific Analyst/Writer, CIR
Date: May 24, 2024
Subject: Wave 2 – Council Comments on the Revised Draft Report on Fatty Amphocarboxylates

Comments on the Revised Draft Report have been received from Council. These comments may be found herein as *PCPCcomments_FattyAmphocarboxylates_Wave2_062024*. It should be noted that these comments state that the submission "Expert Review of Available Repeat-Dose and Developmental and Reproductive Toxicity (DART) Studies for Amphoacetates" by DeSesso and Williams should have been included in the June 2024 packet for this ingredient group. This submission was not included in the June 2024 packet as it was previously submitted and briefly discussed at the June 2023 meeting (as indicated by the transcripts from the June 2023 meeting).

Comments were also received from the REACH Amphoacetates Consortium (*Consortiumcomments_FattyAmphocarboxylates_Wave2_062024).* Please note, these comments suggest the usage of C12 alkylamidopropylbetaine (lauramidopropyl betaine) as read-across for dermal absorption data. (The submitters acknowledge that the Panel previously rejected the use of alkylamidopropyl betaine surfactants (AAPBs) for use as read-across, but are asking the Panel to re-consider as both amphoacetates and AAPBs are ionized at all pH levels).

In addition, read-across justification tables for representative mono- and diacetate forms of alkylamphoacetates, minor constituents, and *N*-(2-hydroxyethl)-*N*-[2-[(1-oxooctyl)amino]ethyl]-β-alanine were previously submitted to the Read-Across Working Group (RAWG) for analysis prior to the June meeting. These documents, including the memo sent to the RAWG, have been included herein for your review as *RA_FattyAmphocarboxylates_Wave2_062024*. This is being included in Wave 2 simply as a source of information, as the RAWG will be reviewing these potential read-across ingredients, along with C12 alkylamidopropylbetaine, during its meeting to determine if they are appropriate for inclusion in the report. The team members will report their findings during Team deliberations.

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: May 21, 2024

SUBJECT: Revised Draft Report: Safety Assessment of Fatty Amphocarboxylates as Used in Cosmetics (draft prepared for the June 2024 meeting)

The Personal Care Products Council respectfully submits the following comments on the revised draft report, Safety Assessment of Fatty Amphocarboxylates as Used in Cosmetics.

Key Issue

Since the Expert Panel for Cosmetic Ingredient Safety is concerned about potential reproductive and developmental toxicity of these ingredients, the assessment by John M. DeSesso, Ph.D., Fellow ATS and Amy Lavin Williams, Ph.D., DABT provided in Wave 2 of the June 2023 meeting (starts on p. 97 of https://www.cir-safety.org/sites/default/files/w2_FA_1.pdf) should have been included in the CIR report, or at least mentioned in the memo. Unpublished analysis/opinions of experts should not automatically be excluded from CIR reports. The Expert Panel should decide whether information from experts should be cited in CIR reports.

Based on analysis of all available developmental and reproductive toxicity data, the DeSesso and Lavin Williams report concludes that "the available developmental and reproductive toxicity data for the four subject amphoacetates do not support the classification of these substances as reproductive or developmental hazard." They also concluded that the impurity (starting material) aminoethylethanolamine (AEEA) could not be responsible for the observed defects.

Developmental and Reproductive Toxicity – Please revise the following to include numbers as only a few fetuses were affected. "Severe cardiac abnormalities were observed in fetuses in all test groups (not including control)". Without numbers, the reader may imply that all the fetuses had severe cardiac abnormalities. According to Table 8, two fetuses in the low dose group had heart malformations (it does not state if the fetuses were from the same or different litters) (transposition of the great vessels in one; situs inversus, interrupted aortic arch and ventricular septum defect in the other), one fetus of the mid-dose group had heart defects (ventricular septum defect, absence of the ductus arteriosus, situs in versus), and one fetus from the high dose

group had heart defects (right-sided aortic arch and ventricular septum defect). Only right-sided aortic arch incidence was above control levels.

Additional Considerations

Table 8 – In the description of the results of the study on Disodium Cocoamphodiacetate (purity 48%), please indicate whether the two fetuses in the low dose group with heart defects were from the same litter.

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

FROM: REACH Amphoacetates Consortium

DATE: May 21, 2024

SUBJECT: Revised Draft Report: Safety Assessment of Fatty Amphocarboxylates as Used in Cosmetics (draft prepared for the June 2024 meeting)

Thank you for your consideration of the following updates and comments on the revised draft report, Safety Assessment of Fatty Amphocarboxylates as Used in Cosmetics.

1. **Status of additional DART studies:** The OECD 414 rabbit prenatal developmental toxicity study (PNDT) on Disodium cocoamphodiacetate is complete and the final draft version of the report is currently under review. The final version should be released by the test lab within the next few weeks, but will not be available by the June 3-4 CIR meeting. We are also planning to have Exponent conduct an independent expert review of this study to be consistent with their previous review of the rat DART data. The in life-phase of the extended one-generation reproductive toxicity study (EOGRTS) on Disodium cocoamphodiacetate was complete at the end of April. The draft report is scheduled for release from the test facility in Sept/Oct this year.

2. **Reason for additional DART studies (questioned in the last expert panel meetings from June 2023):** There was some uncertainty in the CIR report (due to insufficient knowledge of the REACH requirements) as to why we would conduct additional DART studies when we already have the OECD 414 for Disodium cocoamphodiacetate in rats. The REACH amphoacetates consortium would recommend adding a statement in the CIR report that these are standard information requirements for the registered tonnage band, so the additional TG414 studies (in rats and rabbit) and the ongoing TG443 study were neither voluntarily nor driven by the results of the initial OECD TG414 in rats on Disodium cocoamphodiacetate. Rather, they were driven by ECHA challenging the read-across approach and requesting these studies due to the registered tonnage bands for these substances.

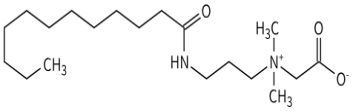
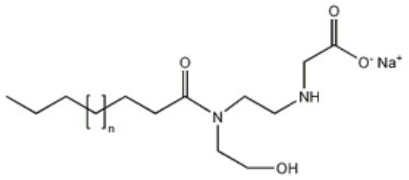
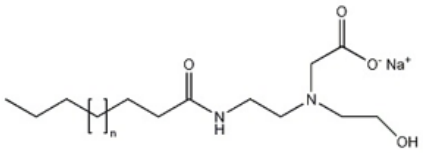
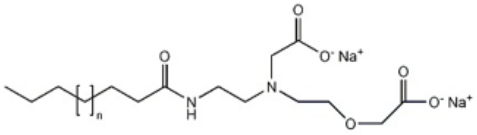
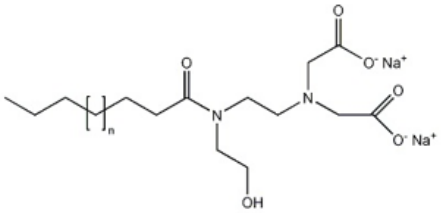
3. **Dermal absorption:** The EU REACH dossiers for C12, C12-14 and C8-18 amphoacetates all rely on read-across to C12 alkylamidopropylbetaine (lauramidopropyl betaine) for dermal absorption data for the human health risk assessment (as summarised in the TK section of the publicly available REACH dossiers). The dermal absorption of lauramidopropyl betaine was <3.5% in wistar han rat but a conservative value of 10% was selected for the risk assessment of this substance and for CAPB (as per the HERA 2005 risk assessment report and the respective REACH

registration dossiers for alkylamidopropyl betaine surfactants). This read-across from lauramidopropyl betaine to amphoacetates was not accepted by the CIR expert panel due to structural differences and concern that the dermal absorption of AAPBs is negligible because they are ionised at all pH levels. The REACH amphoacetates consortium would like to point out that also Amphoacetates are fully ionised at all pH levels, so the read-across might indeed make sense from this perspective. We stand by this read-across within the REACH registration dossiers for the dermal absorption endpoint, as summarised in the read-across justification report previously shared with the CIR expert panel:

Toxicokinetics

An assessment of the toxicokinetic behaviour of alkylamphoacetates C8-C18, alkylamphoacetates C12-14 and alkylamphoacetates C12 to the extent that can be derived from the relevant available information has been performed in accordance with ECHA Guidance on information requirements and chemical safety assessment, Chapter R.7c (May 2008). Oral, dermal and inhalation absorption rates of 100%, 10% and 100% were estimated for each of the routes, respectively. Slight variations observed in the liver weights and/or clinical chemistry in the 28-day repeated dose toxicity study (OECD 407) with alkylampho(mono)acetates C8-C18 and the 90-day repeated dose (OECD 408) studies with alkylampho(di)acetates C12-C14 and alkylampho(di)acetates C8-C18, provided evidence of absorption by the oral route. The dermal absorption rate of 10% is supported by experimental data on a structurally related amphoteric surfactant, dodecylamidopropylbetaine (CAS# 4292-10-8) showing a dermal absorption of less than 3.5% in Wistar rats (HERA 2005). All major constituents of alkylamphoacetates C8-C18, alkylamphoacetates C12-14 and alkylamphoacetates C12 are ionized at all physiological pH levels due to their amphoteric nature, which influences the ability to cross hydrophobic membrane barriers such as skin (WHO, 2006); based on this, 10 % dermal absorption can be considered a highly conservative assumption.

Furthermore, the structural and compositional differences between the source and target(s) are not so significant in our view that it would lead to >10% dermal absorption for amphoacetates. ECHA has also not challenged this assumption for the occupational, professional and consumer risk assessments we have conducted for amphoacetates

Source Lauramidopropyl Betaine	**Targets** C12, C12-14 and C8-18 amphoacetates
Representative structure: 	**Representative structures:** Alkyl-monoacetate 1  Alkyl-monoacetate 2  Alkyl-diacetate 1  Alkyl-diacetate 2 

Cosmetic Ingredient Review

Commitment & Credibility since 1976

Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Read-Across Working-Group Members
From: Jinqiu Zhu, PhD, DABT, ERT, DCST, CIR Toxicologist
Priya Cherian, M.S., Senior Scientific Analyst/Writer
Date: May 10, 2024
Subject: Read-across source materials for Fatty Amphocarboxylates

At the June 2023 meeting, read-across proposals were submitted to the Panel in Wave 2 (https://www.cir-safety.org/sites/default/files/w2_FA_1.pdf). These documents included data and REACH dossiers on various fatty acid chain mixtures (amphoacetates C8-C18 (EC No. 931-291-0), amphoacetates C12-14 (EC No. 938-645-3), and amphoacetates C12 (EC No. 271-794-6)) that comprise ingredients reviewed in the fatty amphocarboxylates report.

In the REACH submission (*Alkylamphoacetate Consortium. 2023. Analogue Approach for REACH Registration of*

Alkylamphoacetates-version December 2022; starting at pdf p45 of the Wave 2 submission) there is some conflicting information regarding the dominant chemical composition of the read-across source materials provided in the above Wave 2 submission (labelling of Figure 1 on pdf p55 of the Wave 2 submission). In concordance with both the *Dictionary* and within the Wave 2 submission itself (pp 90-91), it seems that the structures for "Alkyl-diacetate 1" and "Alkyl-diacetate 2" have been inadvertently swapped. In other words, the primary chemical constituents of the read-across source materials therein *do not* comprise an ethanol substitution at the amide nitrogen. The read-across justification tables included herein (*RA_Table_1_FattyAmphocarboxylates_062024* through *RA_Table_5_FattyAmphocarboxylates_062024*), then, utilize those source structures without an ethanol substitution at the amide nitrogen. A table of relevant properties of the minor constituents of those read-across sources is also provided for completeness (*RA_Table_A_minor-constituents_FattyAmphocarboxylates_062024*). These tables display representative read-across source derivatives for comparing the physicochemical and toxicological properties with the target ingredients. Also note, since coco-derived ingredients are mixtures comprising amphocarboxylates of various chain lengths, with the vast majority falling between C8 and C18, the following justification tables depict the shortest and longest examples of that range for comparison (e.g., there are 2 structural entries for Sodium Cocoamphoacetate, sodium C8-amphoacetate and sodium C18-amphoacetate).

Wave 2 submissions from the June 2023 meeting also included dossiers on the following substances:

- reaction products of 1H-imidazole-1-ethanol, 4-5-dihydro-, 2-(C11-17 and C17 unsatd. alkyl) derivs. (this test substance is equivalent to Disodium Cocoamphodipropionate; therefore, it is included in the report as the ingredient, and is not provided in these read-across tables)
- reaction products of sodium hydroxide and 2-propenoic acid and *N*-(2-hydroxyethl)-*N*-[2-[(1-oxooctyl)amino]ethyl]-β-alanine (as the source analog for the target ingredient of Sodium Cocoamphopropionate, and possibly Sodium Cocoamphoacetate)

Upon review of these submissions, the Read-Across Working Group (RAWG) should consider whether these test substances could potentially serve as read-across sources to target several of the ingredients reviewed in the report.

The proposed correlations of the test substances to the related INCI ingredients included in the report can be found below.

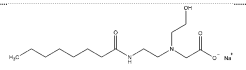
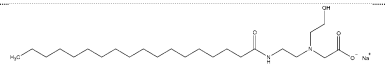
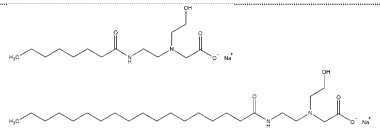
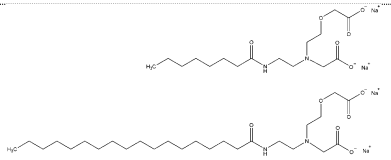
Test Substance Name (proposed sources)	**Related INCI Ingredient (proposed targets)**
amphoacetates C8-18	Disodium Cocoamphodiacetate; Sodium Cocoamphoacetate
amphoacetates C12-C14	Disodium Lauroamphodiacetate; Sodium Lauroamphoacetate
amphoacetates C12	Disodium Lauroamphodiacetate; Sodium Lauroamphoacetate
N-(2-hydroxyethl)-*N*-[2-[(1-oxooctyl)amino]ethyl]-β-alanine	Sodium Cocoamphopropionate; Sodium Cocoamphoacetate

It should be noted that while the suggested additional ECHA dossiers contain information on a variety of endpoints, data on many of these endpoints have already been provided in the Revised Draft Report (and may actually be the same data cited in the proposed ECHA dossier). At the June 2023 meeting, the Panel decided to table the report for organization, noting that the following data (none of which has been received) are needed:

- Dermal absorption data
- DART data on Disodium Cocoamphodiacetate
- Further information regarding the composition and impurities of these ingredients as cosmetics (particularly percentage of actives in ingredients and fatty acid compositions)
- Sensitization data on Sodium Lauroamphoacetate at maximum use concentration

Thus, the RAWG may want to consider if their read-across analyses mitigate any of these data gaps.

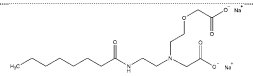
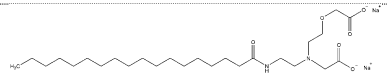
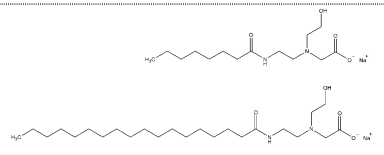
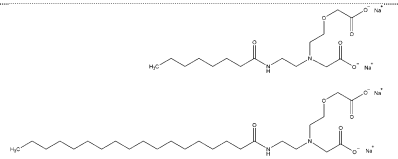
Table 1. Read-across justification: Sodium Cocoamphoacetate - Amphoacetates C8-18 (monoacetate and diacetate)

	Target Ingredients		**Source Analogs**	
Name	***Sodium Cocoamphoacetate (C8)***	***Sodium Cocoamphoacetate(C18)***	***Amphoacetate C8-C18 (monoacetate2)***	***Amphoacetate C8-C18 (diacetate2)***
CAS No.	90387-76-1		—	
Structure				
Tanimoto score **(ChemMine Tools)**	1 *Sodium Cocoamphoacetate (C8) vs. Amphoacetate C8 (monoacetate2)* 0.618 *Sodium Cocoamphoacetate (C8) vs. Amphoacetate C8 (diacetate2)*	1 *Sodium Cocoamphoacetate(C18) vs. Amphoacetate C18 (monoacetate2)* 0.738 *Sodium Cocoamphoacetate(C18) vs. Amphoacetate C18 (diacetate2)*		
Read-across endpoint(s)	• Acute toxicity • Repeated dose toxicity • DART • Genotoxicity • Dermal irritation/sensitization • Ocular irritation			
Formula	$C_{14}H_{27}N_2O_4Na$	$C_{24}H_{47}N_2O_4Na$	$C_{14}H_{27}N_2O_4Na$ - $C_{24}H_{47}N_2O_4Na$	$C_{18}H_{32}N_2O_6Na_2$ - $C_{28}H_{52}N_2O_6Na_2$
Formula Weight (Da)	310.37	450.64	310.37 - 450.64	418.45 - 558.72
Melting Point **(°C, MPBPVP v1.43; EpiSuite)**	297.88	349.84	297.88 - 349.84	309.78 - 349.84
log K_{ow} (KOWWIN v1.68 estimate; EpiSuite)	-3.58	1.33	-3.58 - 1.33	-4.68 - 0.23
Water Solubility **(mg/l, @ 25°C, WSKOW v1.42 in EPI Suite)**	5.999e+005	108.4	5.999e+005 - 108.4	1e+006 - 9.355
Repeated dose toxicity				
Repeat dose (HESS)	Not categorized	Not categorized	Not categorized	Not categorized
Irritation (of the respiratory tract) **(Derek Nexus 6.3)**	Mammal-plausible (Alert: Ethanolamine)	Mammal-plausible (Alert: Ethanolamine)	Mammal-plausible (Alert: Ethanolamine)	Mammal-plausible (Alert: Ethanolamine) (C8) -Not identified (C18)
Skin Sensitization				
Protein Binding Alerts for skin sensitization by OASIS	No alert found	No alert found	No alert found	No alert found
Protein Binding by OECD	No alert found	No alert found	No alert found	No alert found
Protein Binding Alerts according to GSH	Not possible to classify	Not possible to classify	Not possible to classify	Not possible to classify

Skin Sensitization prediction (OECD Toolbox v4.2)	Negative	Positive	Negative (C8) – Positive (C18)	Negative (C8) – Positive (C18)
Skin Sensitization prediction (Derek Nexus 6.3)	Mammal- non sensitizer	Mammal- non sensitizer	Mammal- non sensitizer	Mammal- non sensitizer
Genotoxicity				
DNA binding (OECD Toolbox v4.2)	SN1	SN1	SN1	SN1
Carcinogenicity (genotoxicity and non-genotoxicity) alerts (OECD Toolbox v4.2)	No alert found	No alert found	No alert found	No alert found
Carcinogenicity (Derek Nexus 6.3)	Mammal-plausible (Alert: Ethanolamine or aminoethanethiol)	Mammal-plausible (Alert: Ethanolamine or aminoethanethiol)	Mammal-plausible (Alert: Ethanolamine or aminoethanethiol)	No alert found
DNA alerts for Ames, MN, CA by OASIS	No alert found	No alert found	No alert found	No alert found
In vitro Mutagenicity (Ames test) alerts by ISS	No alert found	No alert found	No alert found	No alert found
In vivo mutagenicity (Micronucleus) alerts by ISS	H-acceptor-path3-H-acceptor	H-acceptor-path3-H-acceptor	H-acceptor-path3-H-acceptor	H-acceptor-path3-H-acceptor
Oncologic Classification (OECD Toolbox v4.2)	Not classified	Not classified	Not classified	Not classified
Mutagenicity in vitro (Derek Nexus 6.3)	Bacterium - inactive	Bacterium - inactive	Bacterium - inactive	Bacterium - inactive
Reproductive and developmental toxicity				
ER Binding (OECD Toolbox v4.2)	Non-binder, non-cyclic structure	Non-binder, non-cyclic structure	Non-binder, non-cyclic structure	Non-binder, MW>500
DART scheme (OECD Toolbox v4.2)	Not known precedent DART potential	Not known precedent DART potential	Not known precedent DART potential	Not known precedent DART potential
Metabolism				
Rat liver S9 metabolism simulator and Structural Alerts for Metabolites (OECD Toolbox v4.2)	13 metabolites: 13×No alert found (DNA binding by OASIS) 4×Schiff base formers (DNA binding by OECD) 13× Non binder (Estrogen receptor binding) 4×Schiff base formation (Protein binding by OASIS) 9×High (Class III) (Toxic hazard classification by Cramer) 4×Simple aldehyde (Genotox) (Carcinogenicity alerts by ISS) 1×Alpha-hydroxy and alkoxyacetic acid derivatives (22b) (DART scheme)	13 metabolites: 13×No alert found (DNA binding by OASIS) 4×Schiff base formers (DNA binding by OECD) 13× Non binder (Estrogen receptor binding) 4×Schiff base formation (Protein binding by OASIS) 9×High (Class III) (Toxic hazard classification by Cramer) 4×Simple aldehyde (Genotox) (Carcinogenicity alerts by ISS) 1×Alpha-hydroxy and alkoxyacetic acid derivatives (22b) (DART scheme) 13×No alert found (DNA alerts for AMEs, CA and MNT by OASIS)	13 metabolites: 13×No alert found (DNA binding by OASIS) 4×Schiff base formers (DNA binding by OECD) 13× Non binder (Estrogen receptor binding) 4×Schiff base formation (Protein binding by OASIS) 9×High (Class III) (Toxic hazard classification by Cramer) 4×Simple aldehyde (Genotox) (Carcinogenicity alerts by ISS) 1×Alpha-hydroxy and alkoxyacetic acid derivatives (22b) (DART scheme) 13×No alert found (DNA alerts for AMEs, CA and MNT by OASIS)	1 metabolite: 1×No alert found (DNA binding by OASIS) 1×SN1 (DNA binding by OECD) 1× Non binder (Estrogen receptor binding) 1×No alert found (Protein binding by OASIS) 1×High (Class III) (Toxic hazard classification by Cramer) 1×No alert found (Genotox) (Carcinogenicity alerts by ISS) 1×Not known (DART scheme) 1×No alert found (DNA alerts for AMEs, CA and MNT by OASIS)

	13×No alert found (DNA alerts for AMEs, CA and MNT by OASIS) 13× Inclusion rules not met (Eye irritation/corrosion inclusion rules by BfR) 4×Simple aldehyde (in vitro mutagenicity (Ames test) alerts by ISS) 13×H-acceptor-path3-H-acceptor (in vivo mutagenicity (Micronucleus) alerts by ISS) 4×Skin sensitization Category 1B (Protein binding alerts for skin sensitization according to GHS)	13× Inclusion rules not met (Eye irritation/corrosion inclusion rules by BfR) 4×Simple aldehyde (in vitro mutagenicity (Ames test) alerts by ISS) 13×H-acceptor-path3-H-acceptor (in vivo mutagenicity (Micronucleus) alerts by ISS) 4×Skin sensitization Category 1B (Protein binding alerts for skin sensitization according to GHS)	13× Inclusion rules not met (Eye irritation/corrosion inclusion rules by BfR) 4×Simple aldehyde (in vitro mutagenicity (Ames test) alerts by ISS) 13×H-acceptor-path3-H-acceptor (in vivo mutagenicity (Micronucleus) alerts by ISS) 4×Skin sensitization Category 1B (Protein binding alerts for skin sensitization according to GHS)	1× Inclusion rules not met (Eye irritation/corrosion inclusion rules by BfR) 1×No alert found (in vitro mutagenicity (Ames test) alerts by ISS) 1×H-acceptor-path3-H-acceptor (in vivo mutagenicity (Micronucleus) alerts by ISS) 1× Not possible classified (Keratinocyte gene expression) 1×No alert found (Protein binding alerts for skin sensitization according to GHS)
Skin metabolism simulator **(OECD Toolbox v4.2)**	1 metabolite: 1×No alert found (DNA binding by OASIS) 1×SN1(DNA binding by OECD) 1× Non binder (Estrogen receptor binding) 1×No alert found (Protein binding by OASIS) 1×High (Class III) (Toxic hazard classification by Cramer)	1 metabolite: 1×No alert found (DNA binding by OASIS) 1×SN1(DNA binding by OECD) 1× Non binder (Estrogen receptor binding) 1×No alert found (Protein binding by OASIS) 1×High (Class III) (Toxic hazard classification by Cramer)	1 metabolite: 1×No alert found (DNA binding by OASIS) 1×SN1(DNA binding by OECD) 1× Non binder (Estrogen receptor binding) 1×No alert found (Protein binding by OASIS) 1×High (Class III) (Toxic hazard classification by Cramer)	1 metabolite: 1×No alert found (DNA binding by OASIS) 1×SN1(DNA binding by OECD) 1× Non binder (Estrogen receptor binding) 1×No alert found (Protein binding by OASIS) 1×High (Class III) (Toxic hazard classification by Cramer)
Justification	Chemical and toxicological properties as well as the compositions of Sodium Cocoamphoacetate and Amphoacetate C8-C18 are expected to be similar. Both the target and source ingredients are a mixture of varying alkyl chain-length (C8-C18) amphoacetates.			

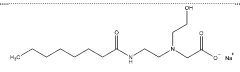
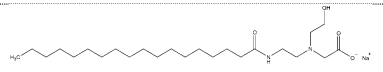
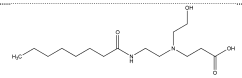
Table 2. Read-across justification: Disodium Cocoamphodiacetate - Amphoacetates C8-18 (monoacetate2 and diacetate2)

	Target Ingredients		**Source Analogs**	
Name	***Disodium Cocoamphodiacetate (C8)***	***Disodium Cocoamphodiacetate (C18)***	***Amphoacetate C8-C18 (monoacetate2)***	***Amphoacetate C8-C18 (diacetate2)***
CAS No.	90387-76-1			
Structure				
Tanimoto score **(ChemMine Tools)**	1 *Disodium Cocoamphodiacetate (C8) vs.Amphoacetate C8 (diacetate2)* 0.618 *Disodium Cocoamphodiacetate (C8) vs. Amphoacetate C8 (monoacetate2)*	1 *Disodium Cocoamphodiacetate(C18) vs. Amphoacetate C18 (diacetate2)* 0.738 *Disodium Cocoamphodiacetate(C18) vs. Amphoacetate C18 (monoacetate2)*		
Read-across endpoint(s)	• Acute toxicity • Repeated dose toxicity • DART • Genotoxicity • Dermal irritation/sensitization • Ocular irritation			
Formula	$C_{18}H_{32}N_2O_6Na_2$	$C_{28}H_{52}N_2O_6Na_2$	$C_{14}H_{27}N_2O_4Na$ - $C_{24}H_{47}N_2O_4Na$	$C_{18}H_{32}N_2O_6Na_2$ - $C_{28}H_{52}N_2O_6Na_2$
Formula Weight (Da)	418.45	558.72	310.37 - 450.64	418.45 - 558.72
Melting Point (°C, MPBPVP v1.43; EpiSuite)	309.78	349.84	297.88 - 349.84	309.78 - 349.84
log K_{ow} (KOWWIN v1.68 estimate; EpiSuite)	-4.68	0.23	-3.58 -1.33	-4.68 - 0.23
Water Solubility (mg/l, @ 25°C, WSKOW v1.42 in EPI Suite)	1e+006	9.355	5.999e+005 - 108.4	1e+006 - 9.355
Repeated dose toxicity				
Repeat dose (HESS)	Not categorized	Not categorized	Not categorized	Not categorized
Irritation (of the respiratory tract) **(Derek Nexus 6.3)**	Mammal-plausible (Alert: Ethanolamine)	Not identified	Mammal-plausible (Alert: Ethanolamine)	Mammal-plausible (Alert: Ethanolamine) (C8) -Not identified (C18)
Skin Sensitization				
Protein Binding Alerts for skin sensitization by OASIS	No alert found	No alert found	No alert found	No alert found
Protein Binding by OECD	No alert found	No alert found	No alert found	No alert found
Protein Binding Alerts according to GSH	Not possible to classify	Not possible to classify	Not possible to classify	Not possible to classify

Skin Sensitization prediction (OECD Toolbox v4.2)	Negative	Positive	Negative (C8) – Positive (C18)	Negative (C8) – Positive (C18)
Skin Sensitization prediction (Derek Nexus 6.3)	Mammal- non sensitizer	Mammal- non sensitizer	Mammal- non sensitizer	Mammal- non sensitizer
Genotoxicity				
DNA binding (OECD Toolbox v4.2)	SN1	SN1	SN1	SN1
Carcinogenicity (genotoxicity and non-genotoxicity) alerts (OECD Toolbox v4.2)	No alert found	No alert found	No alert found	No alert found
Carcinogenicity (Derek Nexus 6.3)	No alert found	No alert found	Mammal-plausible (Alert: Ethanolamine or aminoethanethiol)	No alert found
DNA alerts for Ames, MN, CA by OASIS	No alert found	No alert found	No alert found	No alert found
In vitro Mutagenicity (Ames test) alerts by ISS	No alert found	No alert found	No alert found	No alert found
In vivo mutagenicity (Micronucleus) alerts by ISS	H-acceptor-path3-H-acceptor	H-acceptor-path3-H-acceptor	H-acceptor-path3-H-acceptor	H-acceptor-path3-H-acceptor
Oncologic Classification (OECD Toolbox v4.2)	Not classified	Not classified	Not classified	Not classified
Mutagenicity in vitro (Derek Nexus 6.3)	Bacterium - inactive	Bacterium - inactive	Bacterium - inactive	Bacterium - inactive
Reproductive and developmental toxicity				
ER Binding (OECD Toolbox v4.2)	Non-binder, non-cyclic structure	Non-binder, MW>500	Non-binder, non-cyclic structure	Non-binder, non-cyclic structure (C8) - Non binder, MW>500 (C18)
DART scheme (OECD Toolbox v4.2)	Not known precedent DART potential	Not known precedent DART potential	Not known precedent DART potential	Not known precedent DART potential
Metabolism				
Rat liver S9 metabolism simulator and Structural Alerts for Metabolites (OECD Toolbox v4.2)	1 metabolite: 1×No alert found (DNA binding by OASIS) 1×SN1 (DNA binding by OECD) 1× Non binder (Estrogen receptor binding) 1×No alert found (Protein binding by OASIS) 1×High (Class III) (Toxic hazard classification by Cramer) 1×No alert found (Genotox) (Carcinogenicity alerts by ISS) 1×Not known (DART scheme) 1×No alert found (DNA alerts for AMEs, CA and MNT by OASIS) 1× Inclusion rules not met (Eye irritation/corrosion inclusion rules by	1 metabolite: 1×No alert found (DNA binding by OASIS) 1×SN1 (DNA binding by OECD) 1× Non binder (Estrogen receptor binding) 1×No alert found (Protein binding by OASIS) 1×High (Class III) (Toxic hazard classification by Cramer) 1×No alert found (Genotox) (Carcinogenicity alerts by ISS) 1×Not known (DART scheme) 1×No alert found (DNA alerts for AMEs, CA and MNT by OASIS) 1× Inclusion rules not met (Eye irritation/corrosion inclusion rules by BfR) 1×No alert found (in vitro mutagenicity (Ames test) alerts by ISS)	13 metabolites: 13×No alert found (DNA binding by OASIS) 4×Schiff base formers (DNA binding by OECD) 13× Non binder (Estrogen receptor binding) 4×Schiff base formation (Protein binding by OASIS) 9×High (Class III) (Toxic hazard classification by Cramer) 4×Simple aldehyde (Genotox) (Carcinogenicity alerts by ISS) 1×Alpha-hydroxy and alkoxyacetic acid derivatives (22b) (DART scheme) 13×No alert found (DNA alerts for AMEs, CA and MNT by OASIS) 13× Inclusion rules not met (Eye irritation/corrosion inclusion rules by BfR) 4×Simple aldehyde (in vitro mutagenicity (Ames test) alerts by ISS)	1 metabolite: 1×No alert found (DNA binding by OASIS) 1×SN1 (DNA binding by OECD) 1× Non binder (Estrogen receptor binding) 1×No alert found (Protein binding by OASIS) 1×High (Class III) (Toxic hazard classification by Cramer) 1×No alert found (Genotox) (Carcinogenicity alerts by ISS) 1×Not known (DART scheme) 1×No alert found (DNA alerts for AMEs, CA and MNT by OASIS) 1× Inclusion rules not met (Eye irritation/corrosion inclusion rules by BfR) 1×No alert found (in vitro mutagenicity (Ames test) alerts by ISS)

	BfR) 1×No alert found (in vitro mutagenicity (Ames test) alerts by ISS) 1×H-acceptor-path3-H-acceptor (in vivo mutagenicity (Micronucleus) alerts by ISS) 1× Not possible classified (Keratinocyte gene expression) 1×No alert found (Protein binding alerts for skin sensitization according to GHS)	1×H-acceptor-path3-H-acceptor (in vivo mutagenicity (Micronucleus) alerts by ISS) 1× Not possible classified (Keratinocyte gene expression) 1×No alert found (Protein binding alerts for skin sensitization according to GHS)	13×H-acceptor-path3-H-acceptor (in vivo mutagenicity (Micronucleus) alerts by ISS) 4×Skin sensitization Category 1B (Protein binding alerts for skin sensitization according to GHS)	1×H-acceptor-path3-H-acceptor (in vivo mutagenicity (Micronucleus) alerts by ISS) 1× Not possible classified (Keratinocyte gene expression) 1×No alert found (Protein binding alerts for skin sensitization according to GHS)
Skin metabolism simulator **(OECD Toolbox v4.2)**	1 metabolite: 1×No alert found (DNA binding by OASIS) 1×SN1(DNA binding by OECD) 1× Non binder (Estrogen receptor binding) 1×No alert found (Protein binding by OASIS) 1×High (Class III) (Toxic hazard classification by Cramer)	1 metabolite: 1×No alert found (DNA binding by OASIS) 1×SN1(DNA binding by OECD) 1× Non binder (Estrogen receptor binding) 1×No alert found (Protein binding by OASIS) 1×High (Class III) (Toxic hazard classification by Cramer)	1 metabolite: 1×No alert found (DNA binding by OASIS) 1×SN1(DNA binding by OECD) 1× Non binder (Estrogen receptor binding) 1×No alert found (Protein binding by OASIS) 1×High (Class III) (Toxic hazard classification by Cramer)	1 metabolite: 1×No alert found (DNA binding by OASIS) 1×SN1(DNA binding by OECD) 1× Non binder (Estrogen receptor binding) 1×No alert found (Protein binding by OASIS) 1×High (Class III) (Toxic hazard classification by Cramer)
Justification	Chemical and toxicological properties as well as the compositions of Disodium Cocoamphodiacetate and Amphoacetate C8-C18 are expected to be similar. Both the target and source ingredients are a mixture of varying alkyl chain-length (C8-C18) amphoacetates.			

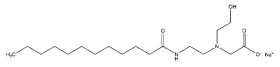
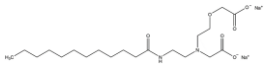
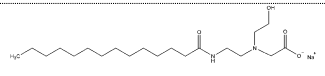
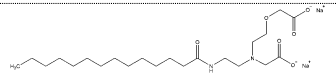
Table 3. Read-across justification: Sodium Cocoamphoacetate - *N*-(2-hydroxyethyl)-*N*-[2-[(oxooctyl)amino]ethyl]-β-alanine

	Target Ingredients		Source Analogs
Name	**Sodium Cocoamphoacetate (C8)**	**Sodium Cocoamphoacetate(C18)**	***N*-(2-hydroxyethyl)-*N*-[2-[(oxooctyl)amino]ethyl]-β-alanine)**
CAS No.	90387-76-1		64265-45-8
Structure			
Tanimoto score **(ChemMine Tools)**	0.955	0.656	
Read-across endpoint(s)	• Acute toxicity • Repeated dose toxicity • DART • Genotoxicity • Dermal irritation/sensitization • Ocular irritation		
Formula	$C_{14}H_{27}N_2O_4Na$	$C_{24}H_{47}N_2O_4Na$	$C_{15}H_{30}N_2O_4$
Formula Weight (Da)	310.37	450.64	302.42
Melting Point **(°C, MPBPVP v1.43; EpiSuite)**	297.88	349.84	311.59
log K_{ow} (KOWWIN v1.68 estimate; EpiSuite)	-3.58	1.33	0.72
Water Solubility **(mg/l, @ 25°C, WSKOW v1.42 in EPI Suite)**	5.999e+005	108.4	2889
Repeated dose toxicity			
Repeat dose (HESS)	Not categorized	Not categorized	Not categorized
Irritation (of the respiratory tract) **(Derek Nexus 6.3)**	Mammal-plausible (Alert: Ethanolamine)	Mammal-plausible (Alert: Ethanolamine)	Mammal-plausible (Alert: Ethanolamine)
Skin Sensitization			
Protein Binding Alerts for skin sensitization by OASIS	No alert found	No alert found	No alert found
Protein Binding by OECD	No alert found	No alert found	No alert found
Protein Binding Alerts according to GSH	Not possible to classify	Not possible to classify	Not possible to classify
Skin Sensitization prediction **(OECD Toolbox v4.2)**	Negative	Positive	Category 1B (indication of skin sensitizing potential) based on GHS criteria
Skin Sensitization prediction **(Derek Nexus 6.3)**	Mammal- non sensitizer	Mammal- non sensitizer	Mammal- non sensitizer
Genotoxicity			
DNA binding **(OECD Toolbox v4.2)**	SN1	SN1	SN1
Carcinogenicity (genotoxicity and non-genotoxicity) alerts **(OECD Toolbox v4.2)**	No alert found	No alert found	No alert found
Carcinogenicity	Mammal-plausible	Mammal-plausible	Mammal-plausible

(Derek Nexus 6.3)	(Alert: Ethanolamine or aminoethanethiol)	(Alert: Ethanolamine or aminoethanethiol)	(Alert: Ethanolamine or aminoethanethiol)
DNA alerts for Ames, MN, CA by OASIS	No alert found	No alert found	No alert found
In vitro Mutagenicity (Ames test) alerts by ISS	No alert found	No alert found	No alert found
In vivo mutagenicity (Micronucleus) alerts by ISS	H-acceptor-path3-H-acceptor	H-acceptor-path3-H-acceptor	H-acceptor-path3-H-acceptor
Oncologic Classification **(OECD Toolbox v4.2)**	Not classified	Not classified	Not classified
Mutagenicity in vitro **(Derek Nexus 6.3)**	Bacterium - inactive	Bacterium - inactive	Bacterium - inactive
Reproductive and developmental toxicity			
ER Binding **(OECD Toolbox v4.2)**	Non binder, non cyclic structure	Non binder, non cyclic structure	Non binder, non cyclic structure
DART scheme **(OECD Toolbox v4.2)**	Not known precedent DART potential	Not known precedent DART potential	Not known precedent DART potential
Metabolism			
Rat liver S9 metabolism simulator and Structural Alerts for Metabolites (OECD Toolbox v4.2)	13 metabolites: 13×No alert found (DNA binding by OASIS) 4×Schiff base formers (DNA binding by OECD) 13× Non binder (Estrogen receptor binding) 4×Schiff base formation (Protein binding by OASIS) 9×High (Class III) (Toxic hazard classification by Cramer) 4×Simple aldehyde (Genotox) (Carcinogenicity alerts by ISS) 1×Alpha-hydroxy and alkoxyacetic acid derivatives (22b) (DART scheme) 13×No alert found (DNA alerts for AMEs, CA and MNT by OASIS) 13× Inclusion rules not met (Eye irritation/corrosion inclusion rules by BfR) 4×Simple aldehyde (in vitro mutagenicity (Ames test) alerts by ISS) 13×H-acceptor-path3-H-acceptor (in vivo mutagenicity (Micronucleus) alerts by ISS) 4×Skin sensitization Category 1B (Protein binding alerts for skin sensitization according to GHS)	13 metabolites: 13×No alert found (DNA binding by OASIS) 4×Schiff base formers (DNA binding by OECD) 13× Non binder (Estrogen receptor binding) 4×Schiff base formation (Protein binding by OASIS) 9×High (Class III) (Toxic hazard classification by Cramer) 4×Simple aldehyde (Genotox) (Carcinogenicity alerts by ISS) 1×Alpha-hydroxy and alkoxyacetic acid derivatives (22b) (DART scheme) 13×No alert found (DNA alerts for AMEs, CA and MNT by OASIS) 13× Inclusion rules not met (Eye irritation/corrosion inclusion rules by BfR) 4×Simple aldehyde (in vitro mutagenicity (Ames test) alerts by ISS) 13×H-acceptor-path3-H-acceptor (in vivo mutagenicity (Micronucleus) alerts by ISS) 4×Skin sensitization Category 1B (Protein binding alerts for skin sensitization according to GHS)	No metabolites
Skin metabolism simulator **(OECD Toolbox v4.2)**	1 metabolite: 1×No alert found (DNA binding by OASIS) 1×SN1(DNA binding by OECD) 1× Non binder (Estrogen receptor binding)	1 metabolite: 1×No alert found (DNA binding by OASIS) 1×SN1(DNA binding by OECD) 1× Non binder (Estrogen receptor binding)	No metabolites

	1×No alert found (Protein binding by OASIS) 1×High (Class III) (Toxic hazard classification by Cramer)	1×No alert found (Protein binding by OASIS) 1×High (Class III) (Toxic hazard classification by Cramer)	
Justification	Chemical and toxicological properties between source analog and target ingredient are expected to be similar. The target ingredient is a mixture of varying alkyl chain-length (C8-C18) amphoacetates and the source chemical is just one length at the shorter end (C8).		

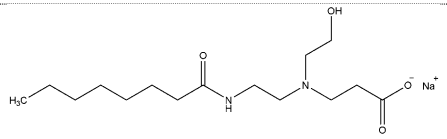
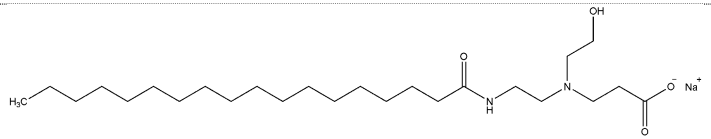
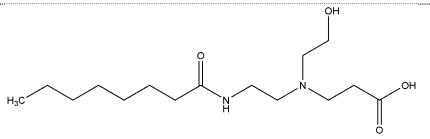
Table 4. Read-across justification: Sodium Lauroamphoacetate/Disodium Lauroamphodiacetate -Amphoacetates C14 (monoacetate2 and diacetate2)

	Target Ingredients		**Source Analogs**	
Name	***Sodium Lauroamphoacetate***	***Disodium Lauroamphodiacetate***	***Amphoacetate C14 (monoacetate2)***	***Amphoacetate C14 (diacetate2)***
CAS No.	90387-76-1	68608-66-2; 156028-14-7; 66161-62-4	—	
Structure				
Tanimoto score **(ChemMine Tools)**	*=0.783* *Sodium Lauroamphoacetate vs. Amphoacetate C14 (monoacetate2)* *=0.727* *Sodium Lauroamphoacetate vs. Amphoacetate C14 (diacetate2)*	*=0.654* *Disodium Lauroamphodiacetate vs. Amphoacetate C14(monoacetate2)* *=0.807* *Disodium Lauroamphodiacetate vs. Amphoacetate C14(diacetate2)*		
Read-across endpoint(s)	• Acute toxicity • Repeated dose toxicity • DART • Genotoxicity • Dermal irritation/sensitization • Ocular irritation			
Formula	$C_{18}H_{35}N_2O_4Na$	$C_{20}H_{36}N_2O_6Na_2$	$C_{20}H_{39}N_2O_4Na$	$C_{22}H_{40}N_2O_6Na_2$
Formula Weight (Da)	366.48	446.50	380.51	474.55
Melting Point (°C, MPBPVP v1.43; EpiSuite)	319.56	320.63	394.53	331.47
log K_{ow} (KOWWIN v1.68 estimate; EpiSuite)	-1.62	-3.70	-0.64	-2.72
Water Solubility (mg/l, @ 25°C, WSKOW v1.42 in EPI Suite)	5810	1.111e+005	566.5	1.07e+004
Repeated dose toxicity				
Repeat dose (HESS)	Not categorized	Not categorized	Not categorized	Not categorized
Irritation (of the respiratory tract) **(Derek Nexus 6.3)**	Mammal-plausible (Alert: Ethanolamine)	No alert found	No alert found	No alert found
Skin Sensitization				
Protein Binding Alerts for skin sensitization by OASIS	No alert found	No alert found	No alert found	No alert found
Protein Binding by OECD	No alert found	No alert found	No alert found	No alert found
Protein Binding Alerts GSH	Not possible to classify	Not possible to classify	Not possible to classify	Not possible to classify
Skin Sensitization prediction (OECD Toolbox v4.2)	Negative	Negative	Negative	Negative
Skin Sensitization prediction (Derek Nexus 6.3)	Mammal- non sensitizer	Mammal- non sensitizer	Mammal- non sensitizer	Mammal- non sensitizer
Genotoxicity				

DNA binding (OECD Toolbox v4.2)	SN1	SN1	SN1	SN1
Carcinogenicity (genotoxicity and non-genotoxicity) alerts (OECD Toolbox v4.2)	No alert found	No alert found	No alert found	No alert found
Carcinogenicity (Derek Nexus 6.3)	Mammal-plausible (Alert: Ethanolamine or aminoethanethiol)	No alert found	Mammal-plausible (Alert: Ethanolamine or aminoethanethiol)	No alert found
DNA alerts for Ames, MN, CA by OASIS	No alert found	No alert found	No alert found	No alert found
In vitro Mutagenicity (Ames test) alerts by ISS	No alert found	No alert found	No alert found	No alert found
In vivo mutagenicity (Micronucleus) alerts by ISS	H-acceptor-path3-H-acceptor	H-acceptor-path3-H-acceptor	H-acceptor-path3-H-acceptor	H-acceptor-path3-H-acceptor
Oncologic Classification (OECD Toolbox v4.2)	Not classified	Not classified	Not classified	Not classified
Mutagenicity in vitro (Derek Nexus 6.3)	Bacterium - inactive	Bacterium - inactive	Bacterium - inactive	Bacterium - inactive
Reproductive and developmental toxicity				
ER Binding (OECD Toolbox v4.2)	Non-binder, non-cyclic structure	Non-binder, non-cyclic structure	Non-binder, non-cyclic structure	Non-binder, non-cyclic structure
DART scheme (OECD Toolbox v4.2)	Not known precedent DART potential	Not known precedent DART potential	Not known precedent DART potential	Not known precedent DART potential
Metabolism				
Rat liver S9 metabolism simulator and Structural Alerts for Metabolites (OECD Toolbox v4.2)	13 metabolites: 13×No alert found (DNA binding by OASIS) 4×Schiff base formers (DNA binding by OECD) 13× Non binder (Estrogen receptor binding) 4×Schiff base formation (Protein binding by OASIS) 9×High (Class III) (Toxic hazard classification by Cramer) 4×Simple aldehyde (Genotox) (Carcinogenicity alerts by ISS) 1×Alpha-hydroxy and alkoxyacetic acid derivatives (22b) (DART scheme) 13×No alert found (DNA alerts for AMEs, CA and MNT by OASIS) 13× Inclusion rules not met (Eye irritation/corrosion inclusion rules by BfR) 4×Simple aldehyde (in vitro mutagenicity (Ames test) alerts by ISS) 2×High gene expression (Keratinocyte gene expression) 13×H-acceptor-path3-H-acceptor (in vivo mutagenicity (Micronucleus) alerts by ISS)	1 metabolite: 1×No alert found (DNA binding by OASIS) 1×SN1 (DNA binding by OECD) 1× Non binder (Estrogen receptor binding) 1×No alert found (Protein binding by OASIS) 1×High (Class III) (Toxic hazard classification by Cramer) 1×No alert found (Genotox) (Carcinogenicity alerts by ISS) 1×Not known (DART scheme) 1×No alert found (DNA alerts for AMEs, CA and MNT by OASIS) 1× Inclusion rules not met (Eye irritation/corrosion inclusion rules by BfR) 1×No alert found (in vitro mutagenicity (Ames test) alerts by ISS) 1×H-acceptor-path3-H-acceptor (in vivo mutagenicity (Micronucleus) alerts by ISS) 1× Not possible classified (Keratinocyte gene expression)	13 metabolites: 13×No alert found (DNA binding by OASIS) 4×Schiff base formers (DNA binding by OECD) 13× Non binder (Estrogen receptor binding) 4×Schiff base formation (Protein binding by OASIS) 9×High (Class III) (Toxic hazard classification by Cramer) 4×Simple aldehyde (Genotox) (Carcinogenicity alerts by ISS) 1×Alpha-hydroxy and alkoxyacetic acid derivatives (22b) (DART scheme) 13×No alert found (DNA alerts for AMEs, CA and MNT by OASIS) 13× Inclusion rules not met (Eye irritation/corrosion inclusion rules by BfR) 4×Simple aldehyde (in vitro mutagenicity (Ames test) alerts by ISS) 2× High gene expression (Keratinocyte gene expression) 13×H-acceptor-path3-H-acceptor (in vivo mutagenicity (Micronucleus) alerts by ISS)	1 metabolite: 1×No alert found (DNA binding by OASIS) 1×SN1 (DNA binding by OECD) 1× Non binder (Estrogen receptor binding) 1×No alert found (Protein binding by OASIS) 1×High (Class III) (Toxic hazard classification by Cramer) 1×No alert found (Genotox) (Carcinogenicity alerts by ISS) 1×Not known (DART scheme) 1×No alert found (DNA alerts for AMEs, CA and MNT by OASIS) 1× Inclusion rules not met (Eye irritation/corrosion inclusion rules by BfR) 1×No alert found (in vitro mutagenicity (Ames test) alerts by ISS) 1×H-acceptor-path3-H-acceptor (in vivo mutagenicity (Micronucleus) alerts by ISS) 1× Not possible classified (Keratinocyte gene expression)

	4×Skin sensitization Category 1B (Protein binding alerts for skin sensitization according to GHS) 4×Aldehydes (Skin irritation/corrosion Inclusion rules by BfR)	1×No alert found (Protein binding alerts for skin sensitization according to GHS)	4×Skin sensitization Category 1B (Protein binding alerts for skin sensitization according to GHS) 4×Aldehydes (Skin irritation/corrosion Inclusion rules by BfR)	1×No alert found (Protein binding alerts for skin sensitization according to GHS)
Skin metabolism simulator **(OECD Toolbox v4.2)**	1 metabolite: 1×No alert found (DNA binding by OASIS) 1×SN1(DNA binding by OECD) 1× Non binder (Estrogen receptor binding) 1×No alert found (Protein binding by OASIS) 1×High (Class III) (Toxic hazard classification by Cramer)	1 metabolite: 1×No alert found (DNA binding by OASIS) 1×SN1(DNA binding by OECD) 1× Non binder (Estrogen receptor binding) 1×No alert found (Protein binding by OASIS) 1×High (Class III) (Toxic hazard classification by Cramer)	1 metabolite: 1×No alert found (DNA binding by OASIS) 1×SN1(DNA binding by OECD) 1× Non binder (Estrogen receptor binding) 1×No alert found (Protein binding by OASIS) 1×High (Class III) (Toxic hazard classification by Cramer) 1× H-acceptor-path3-H-acceptor (in vivo mutagenicity (Micronucleus) alert by ISS	1 metabolite: 1×No alert found (DNA binding by OASIS) 1×SN1(DNA binding by OECD) 1× Non binder (Estrogen receptor binding) 1×No alert found (Protein binding by OASIS) 1×High (Class III) (Toxic hazard classification by Cramer)
Justification	Chemical and toxicological properties between Sodium Lauroamphoacetate, Disodium Lauroamphoacetate, and Amphoacetates C12-C14 or Amphoacetates C12 are expected to be similar. The source analogs are Amphoacetates C12, or Amphoacetates C12-C14.			

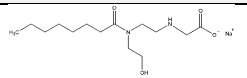
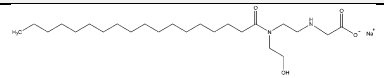
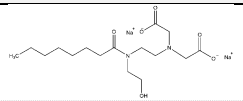
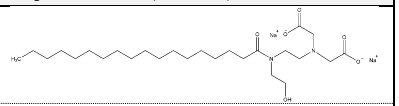
Table 5. Read-across justification: Sodium Cocoamphopropionate - *N*-(2-hydroxyethyl)-*N*-[2-[(oxooctyl)amino]ethyl]-β-alanine)

	Target Ingredients		Source Analog
Name	***Sodium Cocoamphopropionate (C8)***	***Sodium Cocoamphopropionate (C18)***	***N-(2-hydroxyethyl)-N-[2-[(oxooctyl)amino]ethyl]-β-alanine)***
CAS No.	93820-52-1		64265-45-8
Structure			
Tanimoto score (ChemMine Tools)	0.955	0.421	
Read-across endpoint(s)	• Acute toxicity • Repeated dose toxicity • DART • Genotoxicity • Dermal irritation/sensitization • Ocular irritation		
Formula	$C_{15}H_{29}N_2NaO_4$	$C_{25}H_{49}N_2NaO_4$	$C_{15}H_{30}N_2O_4$
Formula Weight (Da)	324.40	464.67	302.42
Melting Point (°C, MPBPVP v1.43; EpiSuite)	303.30	349.84	311.59
log K_{ow} (KOWWIN v1.68 estimate; EpiSuite)	-3.09	1.82	0.72
Water Solubility (mg/l, @ 25°C, WSKOW v1.42 in EPI Suite)	0.00002	33.63	2889
Repeated dose toxicity			
Repeat dose (HESS)	Not categorized	Not categorized	Not categorized
Irritation (of the respiratory tract) **(Derek Nexus 6.3)**	Mammal-plausible (Alert: Ethanolamine)	Mammal-plausible (Alert: Ethanolamine)	Mammal-plausible (Alert: Ethanolamine)
Skin Sensitization			
Protein Binding Alerts for skin sensitization by OASIS	No alert found	No alert found	No alert found
Protein Binding by OECD	No alert found	No alert found	No alert found

Protein Binding Alerts according to GSH	Not possible to classify	Not possible to classify	Not possible to classify
Skin Sensitization prediction (OECD Toolbox v4.2)	Negative	Positive	Category 1B (indication of skin sensitizing potential) based on GHS criteria
Skin Sensitization prediction (Derek Nexus 6.3)	Mammal- non sensitizer	Mammal- non sensitizer	Mammal- non sensitizer
Genotoxicity			
DNA binding (OECD Toolbox v4.2)	SN1	SN1	SN1
Carcinogenicity (genotoxicity and non-genotoxicity) alerts (OECD Toolbox v4.2)	No alert found	No alert found	No alert found
Carcinogenicity (Derek Nexus 6.3)	Mammal-plausible (Alert: Ethanolamine or aminoethanethiol)	Mammal-plausible (Alert: Ethanolamine or aminoethanethiol)	Mammal-plausible (Alert: Ethanolamine or aminoethanethiol)
DNA alerts for Ames, MN, CA by OASIS	No alert found	No alert found	No alert found
In vitro Mutagenicity (Ames test) alerts by ISS	No alert found	No alert found	No alert found
In vivo mutagenicity (Micronucleus) alerts by ISS	H-acceptor-path3-H-acceptor	H-acceptor-path3-H-acceptor	H-acceptor-path3-H-acceptor
Oncologic Classification (OECD Toolbox v4.2)	Not classified	Not classified	Not classified
Mutagenicity in vitro (Derek Nexus 6.3)	Bacterium - inactive	Bacterium - inactive	Bacterium - inactive
Reproductive and developmental toxicity			
ER Binding (OECD Toolbox v4.2)	Non-binder, non-cyclic structure	Non-binder, non-cyclic structure	Non-binder, non-cyclic structure
DART scheme (OECD Toolbox v4.2)	Not known precedent DART potential	Not known precedent DART potential	Not known precedent DART potential
Metabolism			
Rat liver S9 metabolism simulator and Structural Alerts for Metabolites (OECD Toolbox v4.2)	1 metabolite: 1×No alert found (DNA binding by OASIS) 1×SN1 (DNA binding by OECD) 1× Non binder (Estrogen receptor binding)	1 metabolite: 1×No alert found (DNA binding by OASIS) 1×SN1 (DNA binding by OECD) 1× Non binder (Estrogen receptor binding)	No metabolites

	1× No alert found (Protein binding by OASIS) 1×High (Class III) (Toxic hazard classification by Cramer) 1× No alert found (Carcinogenicity alerts by ISS) 1×Not known precedent DART potential 1×No alert found (DNA alerts for AMEs, CA and MNT by OASIS) 1× Inclusion rules not met (Eye irritation/corrosion inclusion rules by BfR) 1×No alert found (in vitro mutagenicity (Ames test) alerts by ISS) 1×H-acceptor-path3-H-acceptor (in vivo mutagenicity (Micronucleus) alerts by ISS) 1×No alert found (Protein binding alerts for skin sensitization according to GHS)	1× No alert found (Protein binding by OASIS) 1×High (Class III) (Toxic hazard classification by Cramer) 1× No alert found (Carcinogenicity alerts by ISS) 1×Not known precedent DART potential 1×No alert found (DNA alerts for AMEs, CA and MNT by OASIS) 1× Inclusion rules not met (Eye irritation/corrosion inclusion rules by BfR) 1×No alert found (in vitro mutagenicity (Ames test) alerts by ISS) 1×H-acceptor-path3-H-acceptor (in vivo mutagenicity (Micronucleus) alerts by ISS) 1×No alert found (Protein binding alerts for skin sensitization according to GHS)	
Skin metabolism simulator (OECD Toolbox v4.2)	1 metabolite: 1×No alert found (DNA binding by OASIS) 1×SN1(DNA binding by OECD) 1× Non binder (Estrogen receptor binding) 1×No alert found (Protein binding by OASIS) 1×H-acceptor-path3-H-acceptor (in vivo mutagenicity (Micronucleus) alerts by ISS) 1×High (Class III) (Toxic hazard classification by Cramer) 1× Inclusion rules not met (Eye irritation/corrosion inclusion rules by BfR) 1×No alert found (Protein binding alerts for skin sensitization according to GHS)	1 metabolite: 1×No alert found (DNA binding by OASIS) 1×SN1(DNA binding by OECD) 1× Non binder (Estrogen receptor binding) 1×No alert found (Protein binding by OASIS) 1×H-acceptor-path3-H-acceptor (in vivo mutagenicity (Micronucleus) alerts by ISS) 1×High (Class III) (Toxic hazard classification by Cramer) 1× Inclusion rules not met (Eye irritation/corrosion inclusion rules by BfR) 1×No alert found (Protein binding alerts for skin sensitization according to GHS)	No metabolites
Justification	Chemical and toxicological properties between source analog and target ingredient are expected to be similar. The target ingredient is a mixture of varying alkyl chain length (C8-C18) amphopropionates and the source chemical is just one length at the shorter end (C8).		

Table A (minor constituents). Amphoacetates C8-18 (monoacetate1 and diacetate1)

	Source Analogs		Source Analogs	
Name	***Amphoacetate C8 (monoacetate1)***	***Amphoacetate C18 (monoacetate1)***	***Amphoacetate C8 (diacetate1)***	***Amphoacetate C18 (diacetate1)***
CAS No.				
Structure				
Tanimoto score **(ChemMine Tools)**	0.624 *Sodium Cocoamphoacetate (C8) vs. Amphoacetate C8 (monoacetate1)*	*0.614* *Sodium Cocoamphoacetate (C18) vs. Amphoacetate C18 (monoacetate1)*	0.559 *Sodium Cocoamphoacetate (C8) vs. Amphoacetate C8 (diacetate1)*	0.687 *Sodium Cocoamphoacetate (C18) vs. Amphoacetate C18 (diacetate1)*
Read-across endpoint(s)*	• Acute toxicity • Repeated dose toxicity • DART • Genotoxicity • Dermal irritation/sensitization • Ocular irritation			
Formula	$C_{14}H_{27}N_2O_4Na$	$C_{24}H_{47}N_2O_4Na$	$C_{16}H_{28}N_2O_6Na_2$	$C_{26}H_{48}N_2O_6Na_2$
Formula Weight (Da)	310.37	450.64	390.39	530.66
Melting Point **(°C, MPBPVP v1.43; EpiSuite)**	283.82	338.02	294.69	348.89
log K_{ow} (KOWWIN v1.68 estimate; EpiSuite)	-3.58	1.33	-6.15	-1.24
Water Solubility **(mg/l, @ 25°C, WSKOW v1.42 in EPI Suite)**	5.999e+005	108.4	1e+006	256.2
Repeated dose toxicity				
Repeat dose (HESS)	Not categorized	Not categorized	1×Ethylenediaminetetracetic acid, EDTA (Renal toxicity) Alert	Not categorized
Irritation (of the respiratory tract) **(Derek Nexus 6.3)**	No alert found	No alert found	No alert found	No alert found
Skin Sensitization				
Protein Binding Alerts for skin sensitization by OASIS	No alert found	No alert found	No alert found	No alert found
Protein Binding by OECD	No alert found	No alert found	No alert found	No alert found
Protein Binding Alerts according to GSH	Not possible to classify	Not possible to classify	Not possible to classify	Not possible to classify
Skin Sensitization prediction **(OECD Toolbox v4.2)**	Negative	Negative	Negative	Positive
Skin Sensitization prediction **(Derek Nexus 6.3)**	Mammal- non sensitizer	Mammal- non sensitizer	Mammal- non sensitizer	Mammal- non sensitizer
Genotoxicity				
DNA binding **(OECD Toolbox v4.2)**	SN1	SN1	SN1	SN1
Carcinogenicity (genotoxicity and non-genotoxicity) alerts **(OECD Toolbox v4.2)**	No alert found	No alert found	No alert found	No alert found
Carcinogenicity **(Derek Nexus 6.3)**	No alert found	No alert found	No alert found	No alert found
DNA alerts for Ames, MN, CA by OASIS	No alert found	No alert found	No alert found	No alert found

In vitro Mutagenicity (Ames test) alerts by ISS	No alert found	No alert found	No alert found	No alert found
In vivo mutagenicity (Micronucleus) alerts by ISS	H-acceptor-path3-H-acceptor	H-acceptor-path3-H-acceptor	H-acceptor-path3-H-acceptor	H-acceptor-path3-H-acceptor
Oncologic Classification (OECD Toolbox v4.2)	Not classified	Not classified	Not classified	Not classified
Mutagenicity in vitro (Derek Nexus 6.3)	Bacterium - inactive	Bacterium - inactive	Bacterium - inactive	Bacterium - inactive
Reproductive and developmental toxicity				
ER Binding (OECD Toolbox v4.2)	Non-binder, non-cyclic structure	Non-binder, non-cyclic structure	Non-binder, non-cyclic structure	Non-binder, MW>500
DART scheme (OECD Toolbox v4.2)	Not known precedent DART potential	Not known precedent DART potential	Not known precedent DART potential	Not known precedent DART potential
Metabolism				
Rat liver S9 metabolism simulator and Structural Alerts for Metabolites (OECD Toolbox v4.2)	5 metabolites: 5×No alert found (DNA binding by OASIS) 1×Schiff base formers (DNA binding by OECD) 5× Non binder (Estrogen receptor binding) 1×Schiff base formation (Protein binding by OASIS) 5×High (Class III) (Toxic hazard classification by Cramer) 1×Simple aldehyde (Genotox) (Carcinogenicity alerts by ISS) 5×Not known (DART scheme) 13×No alert found (DNA alerts for AMEs, CA and MNT by OASIS) 5× Inclusion rules not met (Eye irritation/corrosion inclusion rules by BfR) 1×Simple aldehyde (in vitro mutagenicity (Ames test) alerts by ISS) 5×H-acceptor-path3-H-acceptor (in vivo mutagenicity (Micronucleus) alerts by ISS) 1×Skin sensitization Category 1B (Protein binding alerts for skin sensitization according to GHS)	5 metabolites: 5×No alert found (DNA binding by OASIS) 1×Schiff base formers (DNA binding by OECD) 5×Non binder (Estrogen receptor binding) 1×Schiff base formation (Protein binding by OASIS) 5×High (Class III) (Toxic hazard classification by Cramer) 1×Simple aldehyde (Genotox) (Carcinogenicity alerts by ISS) 5×Not known (DART scheme) 13×No alert found (DNA alerts for AMEs, CA and MNT by OASIS) 5× Inclusion rules not met (Eye irritation/corrosion inclusion rules by BfR) 1×Simple aldehyde (in vitro mutagenicity (Ames test) alerts by ISS) 5×H-acceptor-path3-H-acceptor (in vivo mutagenicity (Micronucleus) alerts by ISS) 1×Skin sensitization Category 1B (Protein binding alerts for skin sensitization according to GHS)	2 metabolites: 2×No alert found (DNA binding by OASIS) 2×SN1 (DNA binding by OECD) 1×Non binder (Estrogen receptor binding) 2×No alert found (Protein binding by OASIS) 2×High (Class III) (Toxic hazard classification by Cramer) 2×No alert found (Genotox) (Carcinogenicity alerts by ISS) 2×Not known (DART scheme) 2×No alert found (DNA alerts for AMEs, CA and MNT by OASIS) 2× Inclusion rules not met (Eye irritation/corrosion inclusion rules by BfR) 2×No alert found (in vitro mutagenicity (Ames test) alerts by ISS) 2×H-acceptor-path3-H-acceptor (in vivo mutagenicity (Micronucleus) alerts by ISS) 2× Not possible classified (Keratinocyte gene expression) 2×No alert found (Protein binding alerts for skin sensitization according to GHS) 1×Ethylenediaminetetracetic acid, EDTA (Renal toxicity) Alert (Repeated dose (HESS))	2 metabolites: 2×No alert found (DNA binding by OASIS) 2×SN1 (DNA binding by OECD) 1× Non binder (Estrogen receptor binding) 2×No alert found (Protein binding by OASIS) 2×High (Class III) (Toxic hazard classification by Cramer) 2×No alert found (Genotox) (Carcinogenicity alerts by ISS) 2×Not known (DART scheme) 2×No alert found (DNA alerts for AMEs, CA and MNT by OASIS) 2× Inclusion rules not met (Eye irritation/corrosion inclusion rules by BfR) 2×No alert found (in vitro mutagenicity (Ames test) alerts by ISS) 2×H-acceptor-path3-H-acceptor (in vivo mutagenicity (Micronucleus) alerts by ISS) 2× Not possible classified (Keratinocyte gene expression) 2×No alert found (Protein binding alerts for skin sensitization according to GHS)
Skin metabolism simulator (OECD Toolbox v4.2)	1 metabolite: 1×No alert found (DNA binding by OASIS) 1×SN1(DNA binding by OECD)	1 metabolite: 1×No alert found (DNA binding by OASIS) 1×SN1(DNA binding by OECD)	1 metabolite: 1×No alert found (DNA binding by OASIS) 1×SN1(DNA binding by OECD)	1 metabolite: 1×No alert found (DNA binding by OASIS) 1×SN1(DNA binding by OECD)

	1× Non binder (Estrogen receptor binding) 1×No alert found (Protein binding by OASIS) 1×High (Class III) (Toxic hazard classification by Cramer) 1×No alert found (in vitro mutagenicity (Ames test) alerts by ISS) 1×H-acceptor-path3-H-acceptor (in vivo mutagenicity (Micronucleus) alerts by ISS)	1× Non binder (Estrogen receptor binding) 1×No alert found (Protein binding by OASIS) 1×High (Class III) (Toxic hazard classification by Cramer) 1×No alert found (in vitro mutagenicity (Ames test) alerts by ISS) 1×H-acceptor-path3-H-acceptor (in vivo mutagenicity (Micronucleus) alerts by ISS)	1×Non binder (Estrogen receptor binding) 1×No alert found (Protein binding by OASIS) 1×High (Class III) (Toxic hazard classification by Cramer) 1×No alert found (in vitro mutagenicity (Ames test) alerts by ISS) 1×H-acceptor-path3-H-acceptor (in vivo mutagenicity (Micronucleus) alerts by ISS) 1×Ethylenediaminetetracetic acid, EDTA (Renal toxicity) Alert (Repeated dose (HESS))	1× Non binder (Estrogen receptor binding) 1×No alert found (Protein binding by OASIS) 1×High (Class III) (Toxic hazard classification by Cramer) 1×No alert found (in vitro mutagenicity (Ames test) alerts by ISS) 1×H-acceptor-path3-H-acceptor (in vivo mutagenicity (Micronucleus) alerts by ISS)
Justification	Chemical and toxicological properties as well as the compositions of Sodium Cocoamphoacetate and the minor constituents of Amphoacetate C8-C18 are expected to be fairly similar. Both the target and minor constituents of the source materials are a mixture of varying alkyl chain-length (C8-C18) amphoacetates.			

Commitment & Credibility since 1976

Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Priya Cherian, MS, Senior Scientific Analyst/Writer, CIR
Date: May 24, 2024
Subject: Wave 2 – Council Comments on the Draft Report on Inositol and Supplier Comments on the Scientific Literature Review

Comments on the Draft Report have been received from Council. These comments may be found herein as *PCPCcomments_Inositol_Wave2_062024*. In addition, comments on the Scientific Literature Review (SLR) were received from a supplier (*Suppliercomments_Inositol_Wave2_062024*) addressing the data requests listed in the SLR. According to these comments, only the isomer *myo*-Inositol is sold by this supplier. The majority of this information (or similar information) has already been provided in the report. Information not already included in the report include the following:

- a clinical study containing 3% *myo*-Inositol; 5-wk use assay; 40 subjects; no skin irritation or skin allergy
- in vitro vaginal irritation assay; up to 16% inositol (isomer not stated); not likely to cause any mucosal irritation
- physical/chemical/heavy metal specifications of *myo*-Inositol (as a cosmetic ingredient)

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: May 21, 2024

SUBJECT: Draft Report: Safety Assessment of Inositol as Used in Cosmetics (draft prepared for the June 2024 meeting)

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

Introduction – myo-Inositol and D-chiro-Inositol are cosmetic ingredients. Therefore, "in the products of cosmetic ingredients" needs to be revised ("ingredients" should be deleted and "cosmetic" should be revised to "cosmetics").

Impurities – "<0.128 ng dioxins" should be corrected (likely ng/kg dioxins based on the units of the other impurities)

Non-Cosmetic Use – The CIR report should state that reference 27 indicates that many of the products are homeopathic drugs. As stated on the labels of these products, FDA does not evaluate homeopathic drugs. The use of Inositol in homeopathic drugs should not be linked to no listing of Inositol in the OTC monographs.

ADME; Summary – The time at which the serum concentrations were measured should be stated. Only groups 1 and 2 had measurements at 24 hours. Only group 1 had a measurement at less than an hour.

Developmental and Reproductive Toxicity – It is not clear what happened to estrus cycles in the Inositol treated mice. It says: "cycles were arrested at day 8-10 in treated mice of all at all concentrations and in positive controls." Since mg/kg doses are stated rather than concentrations, it would be clearer if it said "at all doses of Inositol" (if this is correct).

Sensitization; Summary – How this maximization test was conducted is not clear. It is unlikely that the guinea pigs were "sensitized with 3 intradermal injections". It is more likely that there were 3 groups, and each group was sensitized with a different injection. Seven days after

injection, it was likely that the guinea pigs were treated “epicutaneously” under a closed patch, rather than “intracutaneously” (the injections were the intracutaneous treatments).

Clinical Studies, Effects Observed with Use of Inositol for Disease/Disorder Treatment – Please revise the following sentence: “Studies performed in pregnant women (4 g Inositol (as myo-inositol)/d throughout pregnancy) was not associated....” the sentence structure is saying that “Studies was not associated” – it should likely be that Inositol treatment was not associated with side effects, etc.

Effect of Inositol on Reproductive Dysfunction – Please correct: “[dehy]droepiandrosterone” and “dehydroepiandrost[e]rone” (for both it should be “dehydroepiandrosterone”)

Summary – The duration of the oral study in rats (20-day old and 3-month old rats treated) should be 45 days (as stated in the Short-Term Toxicity section, not “3 mo” as stated in the Summary.

Summary – It should be noted that the trend towards increased mortality was observed in preterm infants (it was in a study of retinopathy of prematurity).

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

FROM: Supplier of Inositol

DATE: May 24, 2024

SUBJECT: Comments on the Inositol Scientific Literature Review (comment period ends May 26, 2024)

Our comments address the data requests listed in the Inositol Scientific Literature Review that was posted on CIR's Website on March 28, 2024.

1. Confirmation of which isomers of Inositol are used in cosmetic formulations (accordingly, all data received should be on a stereoisomer that is used in cosmetics.

   Our company only sells the MYO-INOSITOL isomer (specifications attached).

2. Dermal absorption/dermal penetration data

   We do not have dermal absorption/ penetration data. However, we are including information on endogenous production and daily dietary intake. Endogenous production of inositol in humans amounts to about 4 g/day (about 57 mg/kg bw per day in a 70 kg adult, EFSA 2014). The total dietary intake of inositol in adults is estimated to range between 500 to 1000 mg/day (about 7-14 mg/kg bw per day, EFSA 2014). A review of 12 controlled clinical trials with a total of 250 adults given oral doses of 4 to 30 g inositol/person per day (equal to 57 and 429 mg/kg bw per day for a 70 kg person) over 1 to 12 months, found that the most frequently reported and dose-related adverse effects were nausea, flatulence, loose stools and diarrhoea (Carlomagno and Unfer, 2011). Another study established a maximum tolerated dose (NOAEL level) of 18 g/day (Lam et al., 2006). Inositol is GRAS by the FDA (21 CFR §184.1370). Thus, there is a long history of safe use of inositol as a part of our diet and the amounts are greater than amounts consumers are likely to get exposed to via the cosmetic products.

   References
   a. EFSA. (2014) Scientific Opinion on the safety and efficacy of inositol as a feed additive for fish, dogs and cats. EFSA Journal 12:3671.

b. Carlomagno G., Unfer V. (2011) Inositol safety: Clinical evidences. European Review for Medical and Pharmacological Sciences 15:931-936.
c. Lam S., McWilliams A., LeRiche J., MacAulay C., Wattenberg L., Szabo E. (2006) A phase I study of myo-inositol for lung cancer chemoprevention. Cancer Epidemiol Biomarkers Prev 15:1526-31. DOI: 10.1158/1055-9965.EPI-06-0128.

3. Confirmatory sensitization data at the maximum reported use concentration [2% in face and neck and moisturizing products.

A number of clinical patch tests in the form of Human Repeat Insult Patch Tests (HRIPT) have been conducted with closely related chemical like phytic acid (phosphorylated inositol). The concentration ranges from 0.05-3% have been tested in these studies. No skin irritation or allergic reactions were reported at these concentrations (Cosmetic Ingredient Review report for Polyol phosphates (Release Date: October 18, 2018). Furthermore, we completed a clinical study on a leave on product containing 3% inositol. The studies were completed under the supervision of a dermo-cosmetologist and performed to evaluate the efficacy of Inositol on improving the skin elasticity, used at 3% in a face cream, over a 5 week in-use test on 40 volunteers, against a placebo cream. The efficacy has been evaluated by measuring the skin biomechanical properties, with a cutometer, at Time 0 and after 5 weeks of products application on half face. It can be concluded that the product containing Inositol contributes to improve skin elasticity. Throughout the clinical studies no skin irritation or skin allergy was reported.

The following supplemental information may be of use. In vitro irritation tests have been performed and show that MI is not irritant neither for the eyes, nor for the vaginal mucosa. (see summary below).

Eye Irritation

- Eye irritation test (OECD 492): The eye irritation potential of Inositol was evaluated using the in vitro Epiocular eye irritation test (EIT). The mean tissue viability was >60% when treated with Inositol. Under the test conditions **Inositol is considered as a non-irritant** according to the in vitro Epiocular EIT.

Reference: European Chemicals Agency. Inositol. Accessed June 21, 2022.

Mucosal Irritation

- In vitro vaginal irritation test: the vaginal irritation potential of Inositol was evaluated using an EpiVaginal™ tissue (reconstructed organotypic model representative of human ecto-cervical and vaginal tissue). The experimental design of this study consists of a determination of the direct MTT reduction potential and the pH of the neat liquid test article if possible (and/or dosing solution as appropriate) followed by a definitive assay to determine the ET_{50} value (the exposure time which reduces MTT reduction by 50%). The ET_{50} value are then compared against well-defined, published benchmarks. Inositol was tested at a concentration of 4, 8 and 16% concentration. The ET_{50} was determined to be >24h for all the 3 concentrations tested. These ET_{50} values are comparable to products that are typically applied to this region (example personal lubricants) and are formulated to be non-irritant (Ayehunie S et al, Toxicology in Vitro: 2006 Aug;20(5):689-698). Under the test conditions, **Inositol is not likely to cause any mucosal irritation** based on the in vitro epivaginal irritation test at up to 16% concentration.

Reference: IFF study (2021)

Myo-Inositol – Product Specification
Valid from June 24, 2021

Description
Myo-Inositol For Personal Care

Physical/chemical specifications
Myo-Inositol Content (dry substance)
97 - 100 %
Colour (ICUMSA method) Max. 150
pH, 5% Solution in DiH2O 6.0 - 9.0
Moisture Max. 0.5 % (when packed)
Chloride Max. 50 mg/kg
Sulphate Max. 100 mg/kg
Melting Range 224 - 227 °C
Residue on Ignition 0 - 0.2 %

Microbiological specifications
Total Viable Count Max. 100 CFU/g
Yeasts Max. 100 CFU/g
Molds Max. 100 CFU/g
Total Coliforms NEG /g
E. coli NEG /g
Salmonella NEG /25g

Heavy metal specifications
Heavy metals Max. 10 mg/kg
Arsenic Max. 0.5 mg/kg
Lead Max. 0.5 mg/kg
Iron Max. 5.0 mg/kg

Storage
Stability data is available upon request

Product and manufacturing certifications
- ISO 9001
- Kosher
- ISO 22716

Cosmetic Ingredient Review

Commitment & Credibility since 1976

Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Preethi S. Raj, M.Sc., Senior Scientific Analyst/Writer, CIR
Date: May 24, 2024
Subject: Safety Assessment of *Paeonia suffruticosa*-derived Ingredients as Used in Cosmetics

Please find attached comments received from the Personal Care Products Council on the Draft Report of the Safety Assessment of *Paeonia suffruticosa*-derived Ingredients as Used in Cosmetics.

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: May 21, 2024

SUBJECT: Draft Report: Safety Assessment of *Paeonia suffruticosa*-Derived Ingredients as Used in Cosmetics (draft prepared for the June 2024 meeting)

The Personal Care Products Council respectfully submits the following comments on the draft report, Safety Assessment of *Paeonia suffruticosa*-Derived Ingredients as Used in Cosmetics.

Method of Manufacture, Paeonia Suffruticosa Seed Oil – So it is clear that it is not an essential oil, it would be helpful to identify Paeonia Suffruticosa Seed Oil as a fixed oil in the Method of Manufacture section.

Toxicological Studies, Paeonia Suffruticosa (Tree Peony) Root Bark Extract – In all sections in which studies on the herbal mixture containing 14.29% moutan cortex are described, it would be helpful to state the doses of Paeonia Suffruticosa (Tree Peony) Root Bark Extract in addition to the doses of the mixture.

Tumor Promotion – Since the *Paeonia suffruticosa* preparations did not result in tumor promotion, the title of this section should be changed to Inhibition of Tumor Growth.

Dermal Irritation, Human; Summary – The solvent for the ingredient tested in the 24-hour closed patch study in 20 subjects was 90% ethanol as described on the same summary page that summarizes the irritation study.

Dermal Sensitization, Human; Summary – Since there is enough information to calculate the µg/cm^2 dose used in the HRIPT it should be calculated and stated in the CIR report (0.64 µg root extract/cm^2).

Summary – Since responses vary by cell type, it would be helpful to also state the cell type for which changes in IL-24 levels were described.

Commitment & Credibility since 1976

Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Christina L. Burnett, MSES, Senior Scientific Analyst/Writer, CIR
Date: May 24, 2024
Subject: Wave 2 - Amended Safety Assessment of *p*-Phenylenediamine, *p*-Phenylenediamine HCl, and *p*-Phenylenediamine Sulfate as Used in Cosmetics

Please find attached the comments provided by the Personal Care Products Council on the Draft Final Amended Report on *p*-Phenylenediamine, *p*-Phenylenediamine HCl, and *p*-Phenylenediamine Sulfate (*PCPCcomments_Phenylenediamine_Wave2_062024*).

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: May 21, 2024

SUBJECT: Draft Final Report: Amended Safety Assessment of p-Phenylenediamine, p-Phenylenediamine HCl, and p-Phenylenediamine Sulfate as Used in Cosmetics (draft prepared for the June 2024 meeting)

The Personal Care Products Council respectfully submits the following comments on the draft final report, Amended Safety Assessment of p-Phenylenediamine, p-Phenylenediamine HCl, and p-Phenylenediamine Sulfate as Used in Cosmetics.

Key Issue

Discussion – Is the following sentence necessary in the Discussion? "Furthermore, use of p-Phenylenediamine outside of hair dying products is not within the purview of this Panel." The Discussion does give the Expert Panel's opinion on use of p-Phenylenediamine in temporary tattoos ("highly inappropriate") and in hair dyes applied to the eyebrows and eyelashes ("can result in lost or permanently damaged vision"). These opinions about other uses appears to contradict the statement that use outside of hair dying products is not within the purview of the Expert Panel.

Additional Considerations

Dermal Penetration – In two places it states: "The percutaneous absorption of a commercial [^{14}C]p-Phenylenediamine HCl-containing oxidative hair dye..." . This should be revised as it suggests that the commercial product contains radioactivity. It also should be revised to make it clear that the absorption of p-Phenylenediamine was studied.

ADME, Occupational Studies – Please correct: "Adverse events [were] not reported in any subjects" (add "were")

Acute, old report study – What doses were used in the intraperitoneal and subcutaneous exposure studies?

Developmental and Reproductive – Please correct: "absolute testes weigh[t]" (add "t")

DNA Binding – Add “mice” after $B6C3F_1$

Cytotoxicity, p-Phenylenediamine – How long were the cells exposed? (reference 52)?

Cytotoxicity, p-Phenylenediamine HCl – In the description of reference 53 it states that 10^{-4} cells were treated. Please check this value as 10^{-4} is less than a cell. It is more likely that 10^{4} cells were treated. The units of µg/ml should be called a concentration rather than a dose.

Neurotoxicity, old report summary – Were the “dose-related effects” increases or decreases?

Clinical Studies – How were the patch test results in persons of color different than white patients? (it currently sates “different in a statistically significant manner”).

Commitment & Credibility since 1976

Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Priya Cherian, MS, Senior Scientific Analyst/Writer, CIR
Date: May 24, 2024
Subject: Wave 2 – Additional Data, Comments on the Revised Draft Tentative Report on Prostaglandin Analogues, and Read-Across Justification Tables

Additional data on Isopropyl Cloprostenate (0.0075% in all studies) have been received. These data include the following:

- HET-CAM on mascara and eyeliner containing 0.0075% Isopropyl Cloprostenate; test substance was considered to have "practically no ocular irritation potential" (*data1_ProstaglandinAnalogues_062024_Wave2*)
- Epi-Ocular eye irritation test; test material containing 0.0075% Isopropyl Cloprostenate; non-irritating (*data2_ProstaglandinAnalogues_062024_Wave2*)
- HRIPT; 102 subjects; test material containing 0.0075% Isopropyl Cloprostenate; occlusive conditions; non-irritating and non-sensitizing (grade 1 reaction observed at Challenge 1 for one subject; re-challenge performed) (*data3_ProstaglandinAnalogues_062024_Wave2*)
- HRIPT; 58 subjects; test material containing 0.0075% Isopropyl Cloprostenate; occlusive conditions; non-irritating and non-sensitizing (*data4_ProstaglandinAnalogues_062024_Wave2*)
- HRIPT; 54 subjects; test material containing 0.0075% Isopropyl Cloprostenate; occlusive conditions; non-irritating and non-sensitizing (*data5_ProstaglandinAnalogues_062024_Wave2*)
- Single-center clinical study evaluating the safety and efficacy of a product containing 0.0075% Isopropyl Cloprostenate on eyelashes and eyebrows; 56 subjects; 8 wk of use; adverse effects observed include eye pain, hyperemia, erythema of eyelids, and irritation; no statistically-significant worsening in cutaneous tolerance scores for eyes or eyebrows; no statistically-significant worsening of visual acuity or slit-lamp examination scores including subjective sensations (except fluorescein staining at week 1 on the right eye) (*data6_ProstaglandinAnalogues_062024_Wave2*)
- Safety Assessment: Isopropyl Cloprostenate using Available Data + QSAR Surrogates; submission provides information on and suggests the usage of potential read-across substances (travoprost, latanoprost, and cloprostenol) to fill in data gaps for Isopropyl Cloprostenate (*data7_ProstaglandinAnalogues_062024_Wave2*)

In addition, read-across justification tables were sent to the Read-Across Working Group (RAWG) on May 10, 2024, suggesting the potential use of travoprost and cloprostenol as read-across test substances for Isopropyl Cloprostenate and tafluprost as a read-across test substance for Ethyl Tafluprostamide. Furthermore, the RAWG received an updated read-across table for Isopropyl Cloprostenate that now includes latanoprost, as this ingredient was suggested for read-across in the Safety Assessment: Isopropyl Cloprostenate using Available Data + QSAR Surrogates (*data7_ProstaglandinAnalogues_062024_Wave2).* The updated read-across tables and the memo sent to the RAWG on May 10 have been included herein for your review as *RA_ProstaglandinAnalogues_Wave2_062024*. This is being included in Wave 2 simply as a source of information, as the RAWG will be reviewing these potential read-across ingredients during its meeting to determine if they are appropriate for inclusion in the report. The RAWG team members will report their findings during Team deliberations.

Comments on the Revised Draft Tentative Report have been received and included herein as *PCPCcomments_ProstaglandinAnalogues_Wave2_062024*. These comments note inconsistencies between the data needs

presented in the post-meeting announcement following the December 2023 meeting, and the memo recently sent to the Panel on May 10, 2024. For clarity, the correct insufficiencies are as listed below:

- for Ethyl Tafluprostamide:
  - acute toxicity data
  - repeated dose toxicity data
  - DART data
  - in vivo genotoxicity data

- for Isopropyl Cloprostenate:
  - acute toxicity data
  - repeated dose toxicity data
  - DART data
  - in vitro and in vivo genotoxicity data
  - dermal irritation and sensitization data at the current maximum use concentration of 0.0075%
  - data on local ocular effects (intraocular pressure, iris color change, and periorbital fat loss) at current maximum concentration of use, with independent ophthalmologist to assess colorimetric data regarding iris color change

* Fulfillment of the above data needs was preferred; however, the Panel noted suggestions from industry regarding the use of read-across source to fill in toxicological data gaps for these ingredients, and acknowledged that they would consider confirmatory data (e.g., receptor interaction studies and downstream profiles of adverse effects) to determine if the use of the proposed read-across sources is appropriate to target the ingredients in this report. Lastly, robust information on possible targets and mechanisms regarding these ingredients are requested for both Isopropyl Cloprostenate and Ethyl Tafluprostamide.

Finally, comments were received from 2 companies that manufacture eyelash serums containing 0.0044 or 0.005% Isopropyl Cloprostenate (*Company1&2comments_ProstaglandinAnalogues_Wave2_062024*). As mentioned in these comments, based on additional data received from industry, the EU Commission sent a new mandate to SCCS to further assess the safety of 3 prostaglandin analogues, including Isopropyl Cloprostenate (at up to 0.005%) and Ethyl Tafluprostamide (at up to a 0.018%). More information regarding this mandate can be found at the following link: https://health.ec.europa.eu/document/download/19242ac9-9dc4-451f-ba50-10d3fef7a151_en?filename=sccs2022_q_027.pdf .

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: May 21, 2024

SUBJECT: Revised Draft Tentative Report: Safety Assessment of Ethyl Tafluprostamide and Isopropyl Cloprostenate as Used in Cosmetics (draft prepared for the June 2024 meeting)

The Personal Care Products Council respectfully submits the following comments on the revised draft tentative report, Safety Assessment of Ethyl Tafluprostamide and Isopropyl Cloprostenate as Used in Cosmetics.

Key Issues

IDA Data Needs – The data needs listed in the meeting memo are different than the data needs stated in the post-meeting announcement.

These are the data needs in the memo:
for Ethyl Tafluprostamide:
o acute toxicity data
o repeated dose toxicity data
o developmental and reproductive toxicity data
o in vivo genotoxicity data
o information on targets and mechanisms

for Isopropyl Cloprostenate:
o dermal irritation and sensitization data at the current maximum use concentration of 0.0075%
o data on local ocular effects (intraocular pressure, iris color change) at current maximum concentration of use, with independent ophthalmologist to assess colorimetric data regarding iris color change
o developmental and reproductive toxicity data
o genotoxicity data
o information on targets and mechanisms

These are the data needs as listed in the post-meeting announcement (PMA), and posted on the CIR report status database, and sent to suppliers and PCPC members:

Isopropyl Cloprostenate
• dermal irritation and sensitization data at the current maximum concentration use of 0.0075%
• data on local ocular effects (intraocular pressure, iris color change, and periorbital fat loss) at current maximum concentration of use
o independent ophthalmologist to assess colorimetric data regarding iris color change
• acute toxicity data
• repeated dose toxicity data
• developmental and reproductive toxicity data
• in vitro and in vivo genotoxicity data

Ethyl Tafluprostamide
• acute toxicity data
• repeated dose toxicity data
• developmental and reproductive toxicity data
• in vivo genotoxicity data

If something needs to be changed from the data needs as listed in the PMA, there should be a way to let interested parties know before information is posted for the next meeting.

Somewhere in the report, it would be helpful to discuss the various estimates/studies of dermal absorption of Ethyl Tafluprostamide and why they are so different. The value used in the Margin of Exposure calculation (8.67%) was from a study in which a test formulation containing a use concentration (0.018%) was studied. A dose was calculated using a "conservative dermal absorption of 20%". An in vitro study with Ethyl Tafluprostamide in 50% ethanol (applied at 6 $\mu g/cm^2$) resulted in a dermal penetration of up to 65% at 24 hours.

<u>Additional Considerations</u>
Cosmetic Use; Toxicokinetics – It is not clear why the dose calculation for Isopropyl Cloprostenate is presented in the Toxicokinetics section, while the dose calculation for Ethyl Tafluprostamide is presented in the Cosmetic Use section.

Developmental and Reproductive Toxicity, Parenteral, Isopropyl Cloprostenate – The title of reference 22 indicated they completed electron microscopic examinations. This is not clear from the description of the study in the CIR report.

Ocular Pigmentation, Periorbital Volume, and Adverse Effects, Isopropyl Cloprostenate – Were the adverse effects reported ("include Meibomian gland dysfunction, erythema, corneal epithelial erosion, lid chalazion, conjunctivitis, and tarsal follicles") observed in all subjects?

Endocrine Effects – If available, please state the specific hormonal pathways predicted to be affected. As effects on endocrine pathways can occur without "disruption", please use "endocrine effects", not "endocrine disruption".

Dermal Irritation and Sensitization – Because both ingredients are discussed in the same paragraph, please identify the ingredient in the following sentence. “Three of the four assays [on Isopropyl Cloprostenate containing formulations] were performed under semi-occlusive conditions.”

Risk Assessment; Table 7 – If this risk assessment is left in the report, the studies that provide the points of departure should be described in the CIR report and the references included in the report. The references given in Table 7 (58 and 59) do not appear to be the correct references for the studies.

Summary – Please correct: “reported to Ethyl Tafluprostamide” (“to” should be “for”)

Please revise: “No structural alerts were observed for Ethyl Tafluprostamide according to SAR analyses...” (add “for carcinogenicity” to indicate what structural alerts they were assessing). In the Carcinogenicity section it says that in the additional models examined by SCCS, Ethyl Tafluprostamide and Isopropyl Cloprostenate were “outside the applicability domain”. This needs to be repeated in the Summary.

In the second last paragraph of the Summary, the ingredient in the 0.018% product needs to be stated.

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

FROM: Company 1 (markets cosmetic lash serum containing 0.0044% isopropyl cloprostenate) and Company 2 (markets cosmetic lash serum containing 0.005% isopropyl cloprostenate)

DATE: May 21, 2024

SUBJECT: Revised Draft Tentative Report: Safety Assessment of Ethyl Tafluprostamide and Isopropyl Cloprostenate as Used in Cosmetics (draft prepared for the June 2024 meeting)

We respectfully submit the following comments on the revised draft tentative report, Safety Assessment of Ethyl Tafluprostamide and Isopropyl Cloprostenate as Used in Cosmetics.

In paragraph 2 of the Use Cosmetic section, the report states: “the average concentrations of Isopropyl Cloprostenate in two eyelash serums were determined to be 0.0044 and 0.0048%, respectively,”. This should be changed to 0.0044 and 0.005%, respectively”.

The same sentence in paragraph 2 of the Use Cosmetic section referenced above concludes, “it is unknown if these are marketed serums”. We confirm these are marketed serums.

Footnote 12 in the last sentence of paragraph 2 of the Use Cosmetic section should be removed. This was not included in the Tox Services Report, which deals with eyelash serums containing 0.0044% and 0.005% IC, not 0.0075%. Therefore, the following information from that sentence should also be removed: “(corresponding to 21 ng Isopropyl Cloprostenate per usage of each serum; calculation can be found in the Toxicokinetics Studies section of this report)”.

In the last paragraph of the Use Cosmetic section, the report provides an overview of the SCCS review. On March 27, 2024, based on new data received by industry, the EU Commission sent a new mandate to the Scientific Committee on Consumer Safety (SCCS) to further assess the safety of 3 prostaglandin analogues, including isopropyl cloprostenate up to a max concentration of 0.005% and Ethyl Tafluprostamide up to a max concentration of 0.018%. (see https://health.ec.europa.eu/document/download/19242ac9-9dc4-451f-ba50-10d3fef7a151_en?filename=sccs2022_q_027.pdf) You should update this section to incorporate the new development.

The following language should be removed from the Dermal Absorption section and should be added to the Use Cosmetic section: "The daily exposure to cosmetic eyelash serum is estimated at 0.28 mg,12 and unpublished data submissions indicate that the highest concentration of Isopropyl Cloprostenate in eyelash serum is 0.0075%.11 This results in a daily exposure of 21 ng of Isopropyl Cloprostenate per each use of the lash serum (each use consists of one application to the upper lash line of both eyes)."

The summary of the 8-month clinical on an eyelash serum containing 0.0044% IC in the Ocular Pigmentation, Periorbital Volume and Adverse Events section contains the following inaccuracies and/or omissions. The submitter has included a track changed version of the description for your consideration:

The effect of an eyelash serum containing 0.0044% Isopropyl Cloprostenate on safety and ocular irritation potential, ocular pigmentation and periorbital volume was evaluated in 114 subjects.15,30 Subjects were instructed to apply the serum, once daily, to the clean, dry upper lash line of both eyes, using a single stroke on the eyelid, for 8 mo. Imaging was performed at baseline and at 1, 2, 4, and 8 mo intervals to measure the potential change in ocular pigmentation and periorbital volume. The board-certified ophthalmologist investigator reported no evidence of changes in iris pigmentation or periorbital volume associated with product use as part of the subjects' physical exams over the course of 8 months. For the left iris, there were no statistically-significant differences in red color, green color, or blue color values after all time points of test material use. However, for the right iris, a statistically-significant decrease in green color and blue color was observed with 4 mo of test material use (no differences observed at different time points, or with red color values). After 8 months of usage, there were no statistically significant changes in the RGB color space values of either iris. When compared to baseline, there was a statistically-significant change ~~increase~~ in overall color ~~change~~ Delta-E (absolute value regardless of direction of change) of the left and right iris at all time points. Statistically significant increases in redness values were observed after 8 mo of test material use in the left iris (compared to baseline) and after 4 and 8 mo of test material use in the right iris (compared to baseline). In addition, statistically significant increases in yellowness values were observed in the left iris after 4 and 8 mo of test substance use (compared to baseline) and after 8 mo of test substance use in the right iris (compared to baseline). These changes were determined to be not clinically relevant. When compared to baseline values for the left orbital side, there was a statistically-significant decrease in periorbital volume after 1 mo of use (no difference noted after 2, 4, and 8 mo of use). Similarly, when compared to baseline values for the right orbital side, there was ~~a~~ no statistically-significant difference in the right orbital volume after 2 and 8 mo of test material use. There was no statistically-significant change in periorbital volume in either orbital side after 8 months of usage. Four (4) Adverse Events ~~effects~~ were observed in the study: 1 instance of ~~observed~~ ~~throughout study include~~ bilateral Meibomian gland dysfunction (subject removed from study with all symptoms resolving), 1 instance of early hordeolum/chalazion on the left eye (subject continued study and all symptoms resolved), 1 instance of probable conjunctivitis with punctate epitheliopathy following sand exposure (subject continued study, all symptoms resolved and determined to be unlikely related to test material) and 1 instance of unilateral conjunctivitis (subject removed from study with all symptoms resolving and determined to be possibly test material related) ~~erythema, corneal epithelial erosion, lid chalazion, conjunctivitis, and tarsal follicles~~. The board-certified ophthalmologist investigator determined that the test material was

safe for contact lens and non-contact lens wearers with a slight potential for transient ophthalmological irritation. Ocular irritation evaluated in this study can be viewed in the Ocular Irritation section of this report.

The Case Report section contains the following sentence: "The patient reported the use of an eyelash serum containing Isopropyl Cloprostenate which resulted in irritated periorbital skin after a month of treatment." The sentence should be revised with the following language: "The patient reported the use of an eyelash serum containing an unreported percentage of Isopropyl Cloprostenate which resulted in irritated periorbital skin after a month of treatment."

The Case Report section contains the following sentence: "Periocular effects following the use of an eyelash product containing Isopropyl Cloprostenate were also observed in a 35-yr-old woman who reported use of the product for 10 mo." The sentence should be revised with the following bolded language: "Periocular effects following the use of an eyelash product containing 0.0081% Isopropyl Cloprostenate were also observed in a 35-yr-old woman who reported use of the product for 10 mo."

The Risk Assessment section and the Summary seciton incorrectly cites Tox Services assessment: Tox Services calculated an MOS of 343 for 0.005% IC and did not calculate an MOS for 0.0075% IC. The relevant sentence on page 68 and 70 should be corrected as follows: "An MoS of an eyelash serum containing 0.00~~7~~5% Isopropyl Cloprostenate was calculated to be 343~~228.12~~". If the Panel wishes to use the same values to calculate an MOS for 0.0075% IC, they could add a separate sentence stating: "Using these same values, CIR calculated an MOS of 228 for an eyelash serum containing 0.0075% IC."

The summary of the 8-month clinical in the Summary Section should cross-reference the more detailed description in the Ocular Pigmentation, Periorbital Volume and Adverse Events section.

The conclusion for the micronucleus assay in Table 4 should be "non-clastogenic", not "non-mutagenic".

Cosmetic Ingredient Review

Commitment & Credibility since 1976

Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Read-Across Working-Group Members
From: Jinqiu Zhu, PhD, DABT, ERT, DCST, CIR Toxicologist
Priya Cherian, M.S., Senior Scientific Analyst/Writer, CIR
Date: May 10, 2024
Subject: Read-across source materials for Prostaglandin Analogues

At the December 2023 meeting, data were provided on potential read-across source substances including tafluprost, travoprost, and cloprostenol (*data1_ProstaglandinAnalogues_122023* and *data20_ProstaglandinAnalogues_122023*), along with the submitter's rationale for read-across justification. The use of cloprostenol as a read-across ingredient had previously been rejected by the Panel. However, on April 26, 2024, an additional submission (*data2_ProstaglandinAnalogues_062024*) was received, providing further justification for travoprost and cloprostenol as read-across sources for Isopropyl Cloprostenate. To assist the Read-Across Working-Group (RAWG) in assessing the suitability of these potential source analogs for filling data gaps for the target ingredients, read-across justification tables have been prepared (*RA_Table_1_ProstaglandinAnalogues_062024; RA_Table_2_ProstaglandinAnalogues_062024*). It should be noted in the submissions, points of departure of travoprost and tafluprost have been used in the margin of safety (MOS) calculations for Isopropyl Cloprostenate and Ethyl Tafluprostamide, respectively. Additionally, at the previous meeting, a presentation was delivered on Ethyl Tafluprostamide, which included an additional evaluation of the suitability of tafluprost as a source analog for read-across. A link to this presentation is provided below:

- **PRESENTATION: Safety assessment of Ethyl Tafluprostamide as used in in cosmetic products - Petry & Mishra**
  > Download PDF

At the December 2023 meeting, the Panel issued a second insufficient data announcement (IDA) for these ingredients, and requested the following data:

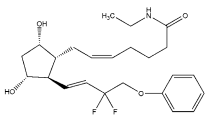
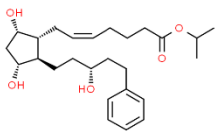
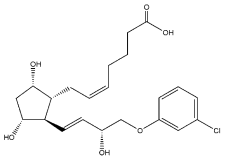
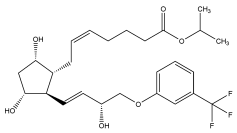
- for Ethyl Tafluprostamide:
  - acute toxicity data
  - repeated dose toxicity data
  - developmental and reproductive toxicity data
  - in vivo genotoxicity data
  - information on targets and mechanisms
- for Isopropyl Cloprostenate:
  - dermal irritation and sensitization data at the current maximum use concentration of 0.0075%
  - data on local ocular effects (intraocular pressure, iris color change) at current maximum concentration of use, with independent ophthalmologist to assess colorimetric data regarding iris color change
  - developmental and reproductive toxicity data

- genotoxicity data
- information on targets and mechanisms

Fulfillment of the above data needs were preferred; however, the Panel noted suggestions from industry regarding the use of read-across sources to fill toxicological data gaps for these prostaglandin ingredients and requested confirmatory data (e.g., receptor interaction studies and downstream profiles of adverse effects) to determine if the use of these read-across sources are appropriate for this report.

The RAWG is requested to review the tables, along with the associated data submissions, and determine the applicability of the potential source substances for conducting read-across for the target prostaglandin ingredients.

Table 1. Read-across justification: Isopropyl Cloprostenate - Latanoprost/Cloprostenol/ Travoprost

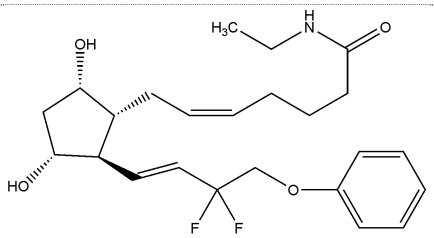
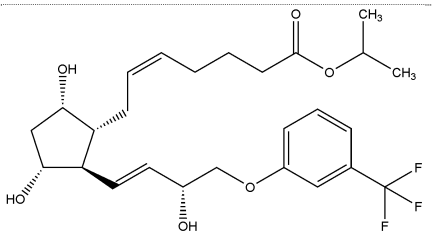
	Target Ingredient	Source Analogs		
Name	**Isopropyl Cloprostenate**	**Latanoprost**	**Cloprostenol**	**Travoprost**
CAS No.	157283-66-4	130209-82-4	54276-21-0	157283-68-6
Structure				
Tanimoto score (ChemMine Tools)	0.623 *Isopropyl Cloprostenate vs. Latanoprost* 0.725 *Isopropyl Cloprostenate vs. Cloprostenol* 0.743 *Isopropyl Cloprostenate vs. Travoprost*			
Read-across endpoint(s)*	• Acute toxicity • Repeated dose toxicity (oral) • DART • Genotoxicity (in vitro and in vivo) •Ocular Irritation • Local ocular effects			
Molecular Formula	$C_{25}H_{35}ClO_6$	$C_{26}H_{40}O_5$	$C_{22}H_{29}ClO_6$	$C_{26}H_{35}F_3O_6$
Molecular Weight (Da)	467.0	432.6	424.9	500.56
Melting Point (°C, MPBPVP v1.43; EpiSuite)	245.38	229.58	253.24	238.24
log K_{ow} (KOWWIN v1.68 estimate; EpiSuite)	5.15	5.67	3.95	0.01532
Water Solubility (mg/l, @ 25°C, WSKOW v1.42 in EPI Suite)	0.04694	0.0274	2.251	5.46
Repeated dose toxicity				
Repeat dose (HESS) **(OECD Toolbox v4.2)**	Not categorized	Not categorized	1×Alpha-Naphthl-isothiocyanate (Hepatotoxicity) Alert	Not categorized
Nephrotoxicology **(Derek Nexus 6.3)**	Mammals-active (Alert: Halogenated benzene)	No alert found	Mammals-active (Alert: Halogenated benzene)	No alert found
Skin Sensitization				
Protein Binding Alerts for skin sensitization by OASIS	No alert found	No alert found	No alert found	No alert found
Protein Binding by OECD	No alert found	No alert found	No alert found	No alert found
Protein Binding Alerts according to GSH	Not possible to classify	Not possible to classify	Not possible to classify	Not possible to classify

Skin Sensitization prediction (OECD Toolbox v4.2)	Positive	Negative	Positive	Positive
Skin Sensitization prediction (Derek Nexus 6.3)	Mammal- active (alert: tertiary allylic hydroperoxide precursor)	Mammal- Non sensitizer	Mammal- active (alert: tertiary allylic hydroperoxide precursor)	Mammal- active (alert: tertiary allylic hydroperoxide precursor)
Genotoxicity				
DNA binding (OECD Toolbox v4.2)	No alert found	Michael addition	No alert found	No alert found
Carcinogenicity (genotoxicity and non-genotoxicity) alerts by ISS (OECD Toolbox v4.2)	Halogenated benzene (Nongenotox)	No alert found	No alert found	No alert found
Carcinogenicity (Derek Nexus 6.3)	No alert found	No alert found	No alert found	No alert found
DNA alerts for Ames, MN, CA by OASIS	No alert found	No alert found	No alert found	No alert found
In vitro Mutagenicity (Ames test) alerts by ISS	No alert found	No alert found	No alert found	No alert found
In vivo mutagenicity (Micronucleus) alerts by ISS	H-acceptor-path3-H-acceptor	No alert found	No alert found	H-acceptor-path3-H-acceptor
Oncologic Primary Classification (OECD Toolbox v4.2)	Halogenated aromatic hydrocarbon type compounds	Not classified	Alpha- and beta-Haloether Reactive Functional groups	Not classified
Mutagenicity in vitro (Derek Nexus 6.3)	Bacterium - inactive	Bacterium - inactive	Bacterium - inactive	Bacterium - inactive
Reproductive and developmental toxicity				
ER Binding (OECD Toolbox v4.2)	Strong binder, OH group	Strong binder, OH group	Strong binder, OH group	Non binder
DART scheme (OECD Toolbox v4.2)	Not known precedent DART potential	Not known precedent DART potential	Not known precedent DART potential	Not known precedent DART potential
Metabolism				
Rat liver S9 metabolism simulator and Structural Alerts for Metabolites (OECD Toolbox v4.2)	14 metabolites: 14×No alert found (DNA binding by OASIS) 5×Michael addition (DNA binding by OECD) 11× Strong binder, OH group (Estrogen receptor binding) 3× Michael addition (Protein binding by OASIS) 11×High (Class III) (Toxic hazard classification by Cramer) 3× alpha,beta-unsaturated carbonyls (genotox) (Carcinogenicity alerts by ISS) 14×Not known (DART scheme) 14×No alert found (DNA alerts for AMEs, CA and MNT by OASIS) 14× Inclusion rules not met (Eye irritation/corrosion inclusion rules by BfR) 3× alpha,beta-unsaturated carbonyls (in vitro	23 metabolites: 23×No alert found (DNA binding by OASIS) 14×Michael addition (DNA binding by OECD) 16× Strong binder, OH group (Estrogen receptor binding) 2× Schiff base formation (Protein binding by OASIS) 14×High (Class III) (Toxic hazard classification by Cramer) 23×No alert found (Carcinogenicity alerts by ISS) 23×Not known (DART scheme) 23×No alert found (DNA alerts for AMEs, CA and MNT by OASIS) 23× Inclusion rules not met (Eye irritation/corrosion inclusion rules by BfR) 23×No alert found (in vitro mutagenicity (Ames test) alerts by ISS)	26 metabolites: 26×No alert found (DNA binding by OASIS) 4×Michael addition (DNA binding by OECD) 20× Strong binder, OH group (Estrogen receptor binding) 2× Michael addition (Protein binding by OASIS) 21×High (Class III) (Toxic hazard classification by Cramer) 2× alpha,beta-unsaturated carbonyls (genotox) (Carcinogenicity alerts by ISS) 26×Not known (DART scheme) 26×No alert found (DNA alerts for AMEs, CA and MNT by OASIS) 26× Inclusion rules not met (Eye irritation/corrosion inclusion rules by BfR) 2× alpha,beta-unsaturated carbonyls (in vitro mutagenicity (Ames test) alerts by ISS) 2× alpha,beta-unsaturated carbonyls (in vivo mutagenicity (Micronucleus) alerts by ISS)	11 metabolites: 11×No alert found (DNA binding by OASIS) 3×Michael addition (DNA binding by OECD) 7× Strong binder, OH group (Estrogen receptor binding) 3× Michael addition (Protein binding by OASIS) 8×High (Class III) (Toxic hazard classification by Cramer) 3× alpha,beta-unsaturated carbonyls (genotox) (Carcinogenicity alerts by ISS) 11×Not known (DART scheme) 11×No alert found (DNA alerts for AMEs, CA and MNT by OASIS) 11× Inclusion rules not met (Eye irritation/corrosion inclusion rules by BfR) 3× alpha,beta-unsaturated carbonyls (in vitro mutagenicity (Ames test) alerts by ISS) 3× alpha,beta-unsaturated carbonyls (in vivo mutagenicity (Micronucleus) alerts by ISS)

	mutagenicity (Ames test) alerts by ISS) 3× alpha,beta-unsaturated carbonyls (in vivo mutagenicity (Micronucleus) alerts by ISS) 4 × very high gene expression (Keratinocyte gene expression) 7×Skin sensitization Category 1B (Protein binding alerts for skin sensitization according to GHS) 1×Aldehyde Type compounds (Oncologic primary classification) 1×1,2- and 1,3-Dicarbonyls (Protein binding potency h-CLAT) 1×AN2 (1×Michael addition to the quinoid type structures)(Protein binding alerts for Chromosomal aberration by OASIS) 1×Acetamide (Renal Toxicity) Alert (Repeated dose (HESS))	13× H-acceptor-path3-H-acceptor (in vivo mutagenicity (Micronucleus) alerts by ISS) 23 × Not possible to classify (Keratinocyte gene expression) 7×Skin sensitization Category 1B (Protein binding alerts for skin sensitization according to GHS) 4×Phenol Type Compounds (Oncologic primary classification) 2×1,2- and 1,3-Dicarbonyls (Protein binding potency h-CLAT) 23×No alert found (Protein binding alerts for Chromosomal aberration by OASIS) 1×Acetamide (Renal Toxicity) Alert (Repeated dose (HESS))	1 × very high gene expression (Keratinocyte gene expression) 2×Skin sensitization Category 1B (Protein binding alerts for skin sensitization according to GHS) 5×Aldehyde Type compounds (Oncologic primary classification) 1×1,2- and 1,3-Dicarbonyls (Protein binding potency h-CLAT) 26×No alert found (Protein binding alerts for Chromosomal aberration by OASIS) 1×Acetamide (Renal Toxicity) Alert (Repeated dose (HESS))	3 × very high gene expression (Keratinocyte gene expression) 7×Skin sensitization Category 1B (Protein binding alerts for skin sensitization according to GHS) 1×Aldehyde Type compounds (Oncologic primary classification) 1×1,2- and 1,3-Dicarbonyls (Protein binding potency h-CLAT) 11×No alert found (Protein binding alerts for Chromosomal aberration by OASIS) 1×Acetamide (Renal Toxicity) Alert (Repeated dose (HESS))
Skin metabolism simulator **(OECD Toolbox v4.2)**	7 metabolites: 7×No alert found (DNA binding by OASIS) 1×Michael addition (DNA binding by OECD) 6× Strong binder, OH group (Estrogen receptor binding) 1× Michael addition (Protein binding by OASIS) 1× alpha,beta-unsaturated-unsaturated ketones (Protein binding potency h-CLAT) 1× Moderately reactive (Protein binding potency GSH) 6×High (Class III) (Toxic hazard classification by Cramer) 1× alpha,beta-unsaturated carbonyls (Carcinogenicity alerts by ISS) 1×Very high gene expression (Keratinocyte gene expression) 6×Halogenated aromatic hydrocarbon type compounds (Oncologic primary classification) 3×Skin sensitization Category 1B (Protein binding alerts for skin sensitization according to GHS) 5×Ketones (Skin irritation/corrosion inclusion rules by BfR)	7 metabolites: 7×No alert found (DNA binding by OASIS) 6×Michael addition (DNA binding by OECD) 6× Strong binder, OH group (Estrogen receptor binding) 7×No alert found (Protein binding by OASIS) 7×No alert found (Protein binding potency h-CLAT) 7×Not possible to classify (Protein binding potency GSH) 3×High (Class III) (Toxic hazard classification by Cramer) 7×No alert found (Carcinogenicity alerts by ISS) 7×Not possible to classify (Keratinocyte gene expression) 7×Not classify (Oncologic primary classification) 3×Skin sensitization Category 1B (Protein binding alerts for skin sensitization according to GHS) 5×Ketones (Skin irritation/corrosion inclusion rules by BfR)	5 metabolites: 5×No alert found (DNA binding by OASIS) 1×Schiff base formers (DNA binding by OECD) 4× Strong binder, OH group (Estrogen receptor binding) 1× Schiff base formation (Protein binding by OASIS) 1× alpha,beta-unsaturated-unsaturated ketones (Protein binding potency h-CLAT) 5×Not possible to classify (Protein binding potency GSH) 4×High (Class III) (Toxic hazard classification by Cramer) 1× Simple aldehyde (genotox) (Carcinogenicity alerts by ISS) 5×Not possible to classify (Keratinocyte gene expression) 1×Aldehyde Type compounds (Oncologic primary classification) 3×Skin sensitization Category 1B (Protein binding alerts for skin sensitization according to GHS) 1×Aldehydes (Skin irritation/corrosion inclusion rules by BfR)	7 metabolites: 7×No alert found (DNA binding by OASIS) 1×Michael addition (DNA binding by OECD) 6× Strong binder, OH group (Estrogen receptor binding) 1× Michael addition (Protein binding by OASIS) 1× alpha,beta-unsaturated-unsaturated ketones (Protein binding potency h-CLAT) 1× Moderately reactive (Protein binding potency GSH) 6×High (Class III) (Toxic hazard classification by Cramer) 1× alpha,beta-unsaturated carbonyls (Carcinogenicity alerts by ISS) 1×Very high gene expression (Keratinocyte gene expression) 1×Reactve ketone reactive functional groups (Oncologic primary classification) 3×Skin sensitization Category 1B (Protein binding alerts for skin sensitization according to GHS) 5×Ketones (Skin irritation/corrosion inclusion rules by BfR)

Justification	Chemical and toxicological properties between Isopropyl Cloprostenate, Cloprostenol and Travoprost are expected to be similar.

Table 2. Read-across justification: Ethyl Tafluprostamide - Tafluprost

	Target Ingredients	Source Analogs
Name	**Ethyl Tafluprostamide**	**Tafluprost**
CAS No.	1185851-52-8	209860-87-7
Structure		
Tanimoto score (ChemMine Tools)	0.536	
Read-across endpoint(s)*		• Acute toxicity • Repeated dose toxicity (oral) • DART • Genotoxicity (in vivo) •Mode of action (targets and mechanism)
Molecular Formula	$C_{24}H_{33}F_2NO_4$	$C_{25}H_{34}F_2O_5$
Molecular Weight (Da)	437.53	452.54
Melting Point (°C, MPBPVP v1.43; EpiSuite)	247.58	204.56
log K_{ow} (KOWWIN v1.68 estimate; EpiSuite)	5.03	6.51
Water Solubility (mg/l, @ 25°C, WSKOW v1.42 in EPI Suite)	0.091	0.0039
Repeated dose toxicity		
Repeat dose (HESS) (OECD Toolbox v4.2)	1×Alpha-Naphthl-isothiocyanate (Hepatotoxicity) Alert	1×Alpha-Naphthl-isothiocyanate (Hepatotoxicity) Alert
Nephrotoxicology (Derek Nexus 6.3)	Mammals-active (Alert: Halogenated benzene)	No alert found
Skin Sensitization		
Protein Binding Alerts for skin sensitization by OASIS	1×Alpha-Naphthl-isothiocyanate (Hepatotoxicity) Alert	No alert found
Protein Binding by OECD	No alert found	No alert found
Protein Binding Alerts according to GSH	Not possible to classify	Not possible to classify
Skin Sensitization prediction (OECD Toolbox v4.2)	Positive	Positive
Skin Sensitization prediction (Derek Nexus 6.3)	Mammal- active (alert: tertiary allylic hydroperoxide precursor)	Mammal- active (alert: tertiary allylic hydroperoxide precursor)
Genotoxicity		
DNA binding (OECD Toolbox v4.2)	No alert found	No alert found
Carcinogenicity (genotoxicity and non-genotoxicity) alerts (OECD Toolbox v4.2)	No alert found	No alert found
Carcinogenicity (Derek Nexus 6.3)	No alert found	No alert found
DNA alerts for Ames, MN, CA by OASIS	No alert found	No alert found No alert found
In vitro Mutagenicity (Ames test) alerts by ISS	No alert found	No alert found
In vivo mutagenicity (Micronucleus) alerts by ISS	No alert found	No alert found

Oncologic Primary Classification **(OECD Toolbox v4.2)**	Alpha- and beta-Haloether Reactive functional groups	Alpha- and beta-Haloether Reactive functional groups
Mutagenicity in vitro **(Derek Nexus 6.3)**	Bacterium - inactive	Bacterium - inactive
Reproductive and developmental toxicity		
ER Binding **(OECD Toolbox v4.2)**	Strong binder, OH group	Strong binder, OH group
DART scheme **(OECD Toolbox v4.2)**	Not known precedent DART potential	Not known precedent DART potential
Metabolism		
Rat liver S9 metabolism simulator and Structural Alerts for Metabolites **(OECD Toolbox v4.2)**	26 metabolites: 26×No alert found (DNA binding by OASIS) 4×Michael addition (DNA binding by OECD) 20× Strong binder, OH group (Estrogen receptor binding) 2× Michael addition (Protein binding by OASIS) 21×High (Class III) (Toxic hazard classification by Cramer) 2× alpha,beta-unsaturated carbonyls (genotox) (Carcinogenicity alerts by ISS) 26×Not known (DART scheme) 26×No alert found (DNA alerts for AMEs, CA and MNT by OASIS) 26× Inclusion rules not met (Eye irritation/corrosion inclusion rules by BfR) 2× alpha,beta-unsaturated carbonyls (in vitro mutagenicity (Ames test) alerts by ISS) 2× alpha,beta-unsaturated carbonyls (in vivo mutagenicity (Micronucleus) alerts by ISS) 1 × very high gene expression (Keratinocyte gene expression) 2×Skin sensitization Category 1B (Protein binding alerts for skin sensitization according to GHS) 5×Aldehyde Type compounds (Oncologic primary classification) 1×1,2- and 1,3-Dicarbonyls (Protein binding potency h-CLAT) 26×No alert found (Protein binding alerts for Chromosomal aberration by OASIS) 1×Acetamide (Renal Toxicity) Alert (Repeated dose (HESS))	10 metabolites: 10×No alert found (DNA binding by OASIS) 2×Michael addition (DNA binding by OECD) 4× Strong binder, OH group (Estrogen receptor binding) 2× Schiff base formation (Protein binding by OASIS) 5×High (Class III) (Toxic hazard classification by Cramer) 1× Simple aldehyde (genotox) (Carcinogenicity alerts by ISS) 10×Not known (DART scheme) 10×No alert found (DNA alerts for AMEs, CA and MNT by OASIS) 10× Inclusion rules not met (Eye irritation/corrosion inclusion rules by BfR) 1× Simple aldehyde (in vitro mutagenicity (Ames test) alerts by ISS) 4× H-acceptor-path3-H-acceptor (in vivo mutagenicity (Micronucleus) alerts by ISS) 1 × very high gene expression (Keratinocyte gene expression) 4×Skin sensitization Category 1B (Protein binding alerts for skin sensitization according to GHS) 1×Aldehyde Type compounds (Oncologic primary classification) 1×1,2- and 1,3-Dicarbonyls (Protein binding potency h-CLAT) 10×No alert found (Protein binding alerts for Chromosomal aberration by OASIS) 1×Acetamide (Renal Toxicity) Alert (Repeated dose (HESS))
Skin metabolism simulator **(OECD Toolbox v4.2)**	5 metabolites: 5×No alert found (DNA binding by OASIS) 1×Schiff base formers (DNA binding by OECD) 4× Strong binder, OH group (Estrogen receptor binding) 1× Schiff base formation (Protein binding by OASIS) 1× alpha,beta-unsaturated-unsaturated ketones (Protein binding potency h-CLAT) 5×Not possible to classify (Protein binding potency GSH) 4×High (Class III) (Toxic hazard classification by Cramer) 1× Simple aldehyde (genotox) (Carcinogenicity alerts by ISS) 5×Not possible to classify (Keratinocyte gene expression)	11 metabolites: 11×No alert found (DNA binding by OASIS) 2×Schiff base formers (DNA binding by OECD) 9× Strong binder, OH group (Estrogen receptor binding) 2× Schiff base formation (Protein binding by OASIS) 2× Monocarbonyls (Protein binding potency h-CLAT) 5×Not possible to classify (Protein binding potency GSH) 4×High (Class III) (Toxic hazard classification by Cramer) 1× Simple aldehyde (genotox) (Carcinogenicity alerts by ISS) 11×Not possible to classify (Keratinocyte gene expression)

	1×Aldehyde Type compounds (Oncologic primary classification) 3×Skin sensitization Category 1B (Protein binding alerts for skin sensitization according to GHS) 1×Aldehydes (Skin irritation/corrosion inclusion rules by BfR)	2×Aldehyde Type compounds (Oncologic primary classification) 6×Skin sensitization Category 1B (Protein binding alerts for skin sensitization according to GHS) 2×Aldehydes (Skin irritation/corrosion inclusion rules by BfR)
Justification	Chemical and toxicological properties of Ethyl Tafluprostamide and Tafluprost are expected to be similar.	

Consumer Product Testing Co.
EST. 1973

FINAL REPORT

CLIENT: [redacted]

ATTENTION: [redacted]

TEST: The Hen's Egg Test - Utilizing the Chorioallantoic Membrane (HET-CAM)

TEST ARTICLE: [redacted] [40-56A 03/06/15]

EXPERIMENT REFERENCE NO.: V15-2816

[redacted]

70 New Dutch Lane • Fairfield, New Jersey 07004-2514 • (973) 808-7111 • Fax (973) 808-7234

Consumer Product Testing Co.
EST. 1975

QUALITY ASSURANCE UNIT STATEMENT

Study No.: V15-2816

The objective of the Quality Assurance Unit (QAU) is to monitor the conduct and reporting of nonclinical laboratory studies. This study has been performed under Good Laboratory Practice principles (including government regulations to the extent applicable) and in accordance with standard operating procedures and applicable standard protocols. The QAU maintains copies of study protocols and standard operating procedures and has inspected this study on the date listed below. The findings of this inspection may have been reported to management and the Study Director.

Date of data inspection: 6/24/15

Quality Assurance:

[redacted]

70 New Dutch Lane • Fairfield, New Jersey 07004-2514 • (973) 808-7111 • Fax (973) 808-7234
Clinical • Toxicology • Analytical Chemistry • Microbiology

Objective:

To evaluate the test article for irritancy potential utilizing the HET-CAM test. The test is a modification of that described by Kemper and Luepke.[1]

Introduction:

The chick embryo has been used extensively in toxicology. "The chorioallantoic membrane (CAM) of the chick embryo is a complete tissue with organoid elements from all germ cell layers. The chorionic epithelium is ectodermal and the allantoic epithelium is endodermal. The mesoderm located between these epithelia is a complete connective tissue including arteries, capillaries, veins and lymphatic vessels. The CAM responds to injury with a complete inflammatory reaction, comparable to that induced in the rabbit eye test. It is technically easy to study, and is without nerves to sense pain."[2]

Test Article: [redacted] [40-56A 03/06/15]

Reference Articles: [redacted] Mascara
[redacted] Eyeliner

Date of Assay: June 19, 2015

The use concentration for IPC in this study is 0.0075%.

[1]Kemper, F.H. & Luepke, N.P., (1986). The HET-CAM Test: An Alternative to the Draize Test. *FD Chem. Toxic.* 24, p. 495 - 496.

[2]Leighton, J., Tchao, R., Verdone, J. & Nassauer, J. Macroscopic Assay of Focal Injury in the Chorioallantoic Membrane. In: *Alternative Methods in Toxicology*, Vol. 3, *In Vitro* Toxicology E2, pp. 357 - 369, Alan M. Goldberg, (ed.), Mary Ann Liebert Publishers, Inc., New York, 1985.

Method:

White Leghorn eggs were obtained from Moyer's Chicks, Inc., in Quakertown, Pennsylvania. For incubation at this facility, the eggs were placed in a Kuhl, humidified incubator. The incubator is such that the eggs are automatically rotated once every hour. The temperature was controlled at 37° C (± 2° C). On day eight (8) the eggs were turned so that the acutely angled end faced down.

On day ten (10) each egg was removed from the incubator and placed in a Plexiglas work enclosure. This enclosure had been preheated and humidified so that its environment approached that of the incubator. A cut was made in the larger end of each egg, where the air sack is located. A Dremel® Moto-Flex Tool (model 232-5) equipped with a Dremel® Cut-Off Wheel (No. 409) was used to make each cut. Forceps were then used to remove the shell down to the shell-membrane junction. The inner egg membrane was then hydrated with a warm, physiological saline solution. The saline was removed after a two (2) to five (5) minute exposure. Utilizing pointed forceps, the inner egg membrane was then carefully removed to reveal the CAM.

The test or reference article, at a dosage of three-tenths of one milliliter (0.3 ml) of a liquid or three-tenths of one gram (0.3 g) of a solid, was then administered to each of four (4) CAM's. Twenty seconds later, the test or reference article was rinsed from each CAM with five (5) milliliters of physiological saline. All CAM's were observed immediately prior to test article administration and at 30 seconds, two (2) and five (5) minutes after exposure to the test article. The reactions of the CAM, the blood vessels, including the capillaries, and the albumin were examined and scored for irritant effects as detailed below:

		Score		
Effect	**Time (min.)**	**0.5**	**2**	**5**
Hyperemia		5	3	1
Minimal Hemorrhage ("Feathering")		7	5	3
Hemorrhage (Obvious Leakage)		9	7	5
Coagulation and/or Thrombosis		11	9	7

The numerical, time dependent scores were totaled for each CAM. Each reaction type can be recorded only once for each CAM, therefore the maximum score per CAM is 32. The mean score was determined for all CAM's similarly tested.

Results:

Test Article (%)	CAM #	Scores @ 0.5 min.	2 min.	5 min.	Total
[redacted]	1	0	0	0	0
[40-56A 03/06/15] (50%)	2	0	3	0	3
	3	0	0	1	1
	4	0	3	0	3
				Average:	**1.75**

Reference Article (%)	CAM #	Scores @ 0.5 min.	2 min.	5 min.	Total
Almay One	1	0	0	1	1
Coat Mascara (50%)	2	0	0	1	1
	3	0	0	0	0
	4	0	0	0	0
				Average:	**0.50**

Reference Article (%)	CAM #	Scores @ 0.5 min.	2 min.	5 min.	Total
Maybelline Waterproof	1	0	0	1	1
Ultra Eyeliner (50%)	2	0	0	1	1
	3	0	0	1	1
	4	0	0	0	0
				Average:	**0.75**

Each article was then classified as indicated in the following:

Mean Score	Irritation Potential
0.0 - 4.9	Practically none
5.0 - 9.9	Slight
10.0 - 14.9	Moderate
15.0 - 32.0	Severe

Discussion:

Previous studies have shown that the CAM of the hen's egg is more sensitive to liquid irritants than is the rabbit eye. Therefore, dilutions of the liquid test and reference articles were used.

Historical *In Vivo* Results:

The reference products have historically been categorized as being practically non-irritating, eliciting scores approaching 0, at 24 hours, when dosed at 100% and tested using the Draize ocular irritation methodologies (Draize Scale: 0 – 110).

Conclusion:

Under the conditions of this test, the results indicate that the sponsor-submitted product, [redacted] [40-56A 03/06/15], at 100%, would have practically no ocular irritation potential *in vivo.*

Record Retention:

All records and documents pertaining to the conduct of this study shall be retained in the CPTC archives for a minimum of ten (10) years. At any time prior to the completion of the tenth archival year, a Sponsor may submit a written request to the CPTC QA Department to obtain custody of study records once the CPTC archive period has been completed. This transfer shall be performed at the Sponsor's expense. In the absence of a written request, study-related records shall be destroyed at the end of the CPTC archive period in a manner that renders them useless.

Professional personnel involved:

[redacted]

- Vice President
  Laboratory Director
  (Study Director)
- Laboratory Supervisor
- Quality Assurance Group Leader

MB Research Labs

Study Title

EpiOcular Eye Irritation Test (EIT)

Test Article

[redacted] F # 72AO-88

Author

[redacted]

Study Completed On

11 May 2020

Performing Laboratory

MB Research Laboratories
1765 Wentz Road
P.O. Box 178
Spinnerstown, PA 18968
phone (215) 536-4110 fax (215) 536-1816

MB Research Project No.

MB 20-27939.19

MB Research Protocol No.

722

Sponsor

[redacted]

MB Research Labs

Study Title : EpiOcular Eye Irritation Test (EIT)
Project No. : 20-27939.19

GOOD LABORATORY PRACTICES COMPLIANCE STATEMENT

This study was conducted in accordance with the Good Laboratory Practice requirements of EPA, 40 CFR 160 and 792, FDA 21 CFR 58, and as specified in Principles on Good Laboratory Practices, published by the Organization for Economic Cooperation & Development (OECD), with the following exception:

The Sponsor did not provide test article characterization information. The effect of the lack of test article characterization information cannot be fully assessed.

STUDY DIRECTOR: [redacted] 11 May 2020
Date

MB RESEARCH LABORATORIES

MB Research Labs

Study Title : EpiOcular Eye Irritation Test (EIT)
Project No. : 20-27939.19

QUALITY ASSURANCE STATEMENT

The Quality Assurance Unit has inspected a critical phase of this study, audited the raw data and the report and determined that the methods and results contained herein accurately reflect the raw data. A summary of the compliance inspections is presented below.

Date of Inspection	Phase	Performed By	Date Inspection Results Reported	
			Study Director	Management
08 Apr 2020	Dose administration	[redacted]	08 Apr 2020	08 Apr 2020
21 Apr 2020	Raw data audit		21 Apr 2020	21 Apr 2020
30 Apr 2020	Draft report audit		30 Apr 2020	06 May 2020
06 May 2020	Final report audit		06 May 2020	06 May 2020

[redacted]

08 May 2020
Date

Quality Assurance Unit

MB Research Labs

Study Title : EpiOcular Eye Irritation Test (EIT)
Project No. : 20-27939.19

TABLE OF CONTENTS

MB Research Labs

Study Title : EpiOcular Eye Irritation Test (EIT)
Project No. : 20-27939.19

KEY PERSONNEL

[redacted]	Chief Scientific Officer
	Director of Toxicology
	Study Director

MB Research Labs

PROJECT NO.	:	**MB 20-27939.19**
SPONSOR	:	[redacted]
TITLE	:	**EpiOcular Eye Irritation Test (EIT)**
PROTOCOL NO.	:	**722**

ABSTRACT

Objective: The purpose of this study was to provide classification of chemicals concerning their eye irritation potential using an alternative to the Draize Rabbit Eye Test, according to the OECD Test Guideline No. 492, "Reconstructed Human Cornea-like Epithelium (RhCE) Test Method for Identifying Chemicals Not Requiring Classification and Labelling for Eye Irritation or Serious Eye Damage". The EpiOcular™ EIT was intended to differentiate those materials that are UN GHS No Category (i.e., do not meet the requirements for UN GHS classification) from those that would require labeling as either UN GHS Category 1 or 2. This assay was not intended to differentiate between UN GHS Category 1 and UN GHS Category 2 (nor between EU R36 and R41).

Method Synopsis: MatTek EpiOcular™ tissues were treated with the test article, negative control and positive control in duplicate for 30 minutes. Following treatment and subsequent incubation time, the viability of the tissues was determined using thiazolyl blue tetrazolium bromide (MTT) uptake and reduction. The absorbance of each sample was measured at 570 nm. The viability was then expressed as a percent of negative control values. If the mean tissue viability was less than or equal to 60%, the test material was classified as an Irritant (UN GHS Category 1 or 2); if the mean tissue viability was greater than 60%, the test material was classified as UN GHS No Category, and was therefore interpreted to be a Non-Irritant.

Summary/Conclusions:

Test and Control Article Identity	Mean Tissue Viability (%)	Irritancy Classification	GHS Classification
[redacted] F # 72AO-88	97.4	Non-Irritant	No Category
Tissue culture water (Negative Control)	100.0	Non-Irritant	No Category
Methyl acetate (Positive Control)	34.9	Irritant	Category 1 or 2

MB Research Labs

Study Title : EpiOcular Eye Irritation Test (EIT)
Project No. : 20-27939.19

OBJECTIVE

The purpose of this study was to provide classification of chemicals concerning their eye irritation potential using an alternative to the Draize Rabbit Eye Test, according to the OECD Test Guideline No. 492, "Reconstructed Human Cornea-like Epithelium (RhCE) Test Method for Identifying Chemicals Not Requiring Classification and Labelling for Eye Irritation or Serious Eye Damage". The EpiOcular™ EIT was intended to differentiate those materials that are UN GHS No Category (i.e., do not meet the requirements for UN GHS classification) from those that would require labeling as either UN GHS Category 1 or 2. This assay was not intended to differentiate between UN GHS Category 1 and UN GHS Category 2 (nor between EU R36 and R41).

TEST ARTICLE

Identity	:	[redacted] F # 72AO-88
Provided by	:	[redacted]
Test Article Characterization	:	Not supplied by the Sponsor
Date Received	:	13 Mar 2020
Storage	:	Room temperature and humidity
Description	:	Opaque black semi-solid
Sample Preparation	:	Used as received

The use concentration for IPC in this study is 0.0075%.

POSITIVE CONTROL

Identity	:	Methyl acetate, Lot No. 030620ALA
Provided by	:	MatTek Corporation
Date Received	:	07 Apr 2020
Expiration Date	:	06 Jun 2020
Storage	:	Room temperature and humidity
Description	:	Clear colorless liquid
Sample Preparation	:	Used as received

NEGATIVE CONTROL

Identity	:	Tissue culture water, (TCH_2O) Lot No. RNBJ0041 (See Appendix A for Certificate of Analysis)
Provided by	:	Sigma-Aldrich
Date Received	:	17 Mar 2020
Expiration Date	:	Nov 2021
Storage	:	Room temperature and humidity
Description	:	Clear colorless liquid
Sample Preparation	:	Used as received

TEST DATES

Study Initiation	(date protocol signed)	:	03 Apr 2020
Experimental Start Date	(1st date data collected – OECD)	:	06 Apr 2020
Experimental Start Date	(1st exposure to test substance)	:	08 Apr 2020
Experimental Term Date	(last date data collected)	:	09 Apr 2020
Draft Report Submitted	(if applicable)	:	05 May 2020
Final Report Signed	(study completion)	:	11 May 2020

EXPERIMENTAL DESIGN

Plate Reader Linearity Check

The linearity of the plate reader used for optical density (OD) determination was verified prior to its use the same week the EIT assay was performed. A dilution series of trypan blue was prepared and two 200-µl aliquots per concentration were pipetted into a 96-well plate. The optical density of the plate wells was measured at a wavelength of 570 nm (OD_{570}), with no reference wavelength. A regression line and an R-squared value were generated using Microsoft Excel® 2007. Verification was considered acceptable if the R-squared value was greater than 0.999.

Test Article Reduction of MTT

A total of 50 µl of the test article were mixed with 1 ml of MTT solution (1 mg/ml methyl thiazole tetrazolium diluted in Dulbecco's Modified Eagle's Medium [DMEM]). A negative control (50 µl of tissue culture water, TCH_2O) was tested concurrently. The solutions were incubated in the dark at 37 ± 1°C, 5 ± 1% CO_2 for 3 hours in a six-well plate. After incubation, the solutions were visually inspected for purple coloration, which indicates that the test article reduced MTT. Since tissue viability is based on MTT reduction, direct reduction by a test article can exaggerate viability, making a test article seem less irritating than its actual irritation potential. The test article did not reduce MTT and the assay continued as per the protocol.

Assessment of Coloring or Staining Materials

The test article was colored; therefore, it was assessed for its ability to absorb light at the wavelength used for MTT determination (570 nm). A total of 50 µl of the test article were added to 2 ml of extractant (isopropanol) and incubated for 2 to 3 hours in a six-well plate, at room temperature with shaking. Two 200-µl aliquots of the test article plus extractant were transferred to a 96-well plate and measured at 570 nm using a plate reader (µQuant Plate Reader, Bio-Tek Instruments, Winooski, VT). The mean OD_{570} value of the test article plus extractant was no more than 0.08 after subtraction of blank (isopropanol), so no colorant controls were performed with the assay.

EXPERIMENTAL DESIGN (continued)

EpiOcular™ Tissue Samples

EpiOcular™ tissues, Lot No. 31733, Kit A, were received from MatTek Corporation (Ashland, MA) on 07 Apr 2020 and refrigerated at 2 to 8°C. See Appendix B for EpiOcular™ tissue Quality Control report. Before use, the tissues were incubated (37 ± 1°C, 5 ± 1% CO_2) with assay medium (MatTek) for a one-hour equilibration. Equilibration medium was replaced with fresh medium for an additional overnight equilibration of 16 to 24 hours. After the overnight incubation, the tissues were moistened with 20 µl of Dulbecco's phosphate-buffered saline (DPBS) and incubated at 37 ± 1°C, 5 ± 1% CO_2 for 30 ± 2 minutes.

Dosing

A total of 50 µl of the test article were applied to EpiOcular™ tissues in duplicate. A negative control (50 µl of TCH_2O) and a positive control (50 µl of methyl acetate) were each tested concurrently. Each treatment with test article or control was conducted in duplicate. The tissues were incubated at 37 ± 1°C, 5 ± 1% CO_2 for 30 ± 2 minutes. After dosing and incubation, the tissues were rinsed with PBS and soaked in 5 ml of room-temperature assay medium in a 12-well plate for 12 minutes. The soaked tissues were then incubated in fresh assay medium at 37 ± 1°C, 5 ± 1% CO_2 for 120 minutes.

Tissue Viability (MTT Reduction)

At the end of the incubation period, each EpiOcular™ tissue was transferred to a 24-well plate containing 300 µl of MTT solution (1 mg/ml MTT in DMEM). The tissues were then returned to the incubator for a three-hour MTT incubation period. Following the MTT incubation period, each EpiOcular™ tissue was rinsed with DPBS and then treated with 2.0 ml of extractant (isopropanol) overnight at room temperature in the dark. Two 200-µl aliquots of the extracted MTT formazan from each well were transferred to a 96-well plate and measured at 570 nm using a plate reader (µQuant Plate Reader, Bio-Tek Instruments, Winooski, VT).

Analysis of Data

See Table 1 for Experimental Data. The mean absorbance value for each time point was calculated from the optical density (OD) of the duplicate samples and expressed as percent viability for each sample using the following formula:

$$\% \text{ viability} = 100 \text{ X } (OD_{sample}/OD_{negative\ control})$$

Quality Controls

The assay meets the acceptance criterion if the mean OD_{570} of the negative control tissues is greater than 0.8 and less than 2.5, and the mean relative viability of positive control tissues, expressed as percentage of the negative control tissues, is less than 50%. In addition, the difference in viability between identically treated tissues must be less than 20%.

EXPERIMENTAL DESIGN (continued)

Ocular Irritation Prediction

According to the OECD[1] Guideline, and GHS[2] classification, an irritant is predicted if the mean relative tissue viability of two individual tissues exposed to the test substance is ≤ 60% of the mean viability of the negative controls.

In Vitro result	GHS Classification
Mean tissue viability less than or equal to 60%	Category 1 or 2
Mean tissue viability greater than 60%	No category

If the test article-treated tissue viability is 60±5%, a second EIT should be performed. If the results of the second test disagree with the first, then a third test should be performed. The conclusion will be based on the agreement of two of the three tests.

Retention of Data

Upon signing the final report, all raw data, supporting documentation and reports are submitted to the Archivist by the Study Director. The raw data are filed at MB Research by project number. The final report is filed at MB Research by Sponsor name and MB Research project number.

All data generated during the conduct of this study are archived at MB Research for at least ten years from the date of the final report. The Sponsor will be contacted in writing to determine final disposition of the records. If the Sponsor fails to respond within 90 days, the archived items will be properly discarded.

Any remaining test article will be discarded upon submission of the report.

Amendment to the Protocol

There were no amendments to the protocol. See Appendix C for the protocol in its entirety.

[1]OECD Guideline for the Testing of Chemicals No. 492: Reconstructed Human Cornea-like Epithelium (RhCE) Test Method for Identifying Chemicals Not Requiring Classification and Labeling for Eye Irritation or Serious Eye Damage.
[2]Globally Harmonized System of Classification and Labeling of Chemicals (GHS). Sixth Revised Edition. United Nations - New York and Geneva. 2015.

MB Research Labs

Study Title : EpiOcular Eye Irritation Test (EIT)
Project No. : 20-27939.19

RESULTS AND CONCLUSIONS

The test article provided by [redacted] was tested using the MatTek EpiOcular™ Eye Irritation Test (EIT).

The summarized results and irritation classifications are as follows:

Test and Control Article Identity	Tissue Viability (%)		Irritancy Classification	GHS Classification
	Mean	Diff.		
[redacted] F # 72AO-88	97.4	1.71	Non-Irritant	No Category
Tissue culture water (Negative Control)	100.0	1.27	Non-Irritant	No Category
Methyl acetate (Positive Control)	34.9	0.84	Irritant	Category 1 or 2

FINAL REPORT

Approved by: [redacted] 11 May 2020
Study Director Date

MB Research Labs

Study Title : EpiOcular Eye Irritation Test (EIT)
Project No. : 20-27939.19

Table 1: Experimental Data

Test and Control Article Identity	Tissue No.	Raw OD data		Blank corrected OD data		Mean of Aliquots	% Viability	OD		Viabilities (%)	
		Aliq. 1	Aliq. 2	Aliq. 1	Aliq. 2			Mean	Diff.	Mean	Diff.
[redacted] F # 72AO-88	1	1.553	1.650	1.507	1.604	1.556	96.5	1.569	0.028	97.4	1.71
	2	1.649	1.609	1.603	1.563	1.583	98.2				
TCH_2O (Negative Control)	1	1.610	1.726	1.564	1.680	1.622	100.6	1.612	0.021	100.0	1.27
	2	1.667	1.628	1.621	1.582	1.602	99.4				
Methyl acetate (Positive Control)	1	0.599	0.603	0.553	0.557	0.555	34.4	0.562	0.014	34.9	0.84
	2	0.618	0.611	0.572	0.565	0.569	35.3				

Diff. = difference between tissues OD = optical density

Blank Data

No.	1	2	3	4	5	6	7	8	Mean
OD	0.045	0.046	0.045	0.045	0.046	0.045	0.047	0.049	**0.046**

Quality Controls:

The mean OD_{570} of the negative control tissues was 1.612, which met the acceptance criteria of greater than 0.8 and less than 2.5. The mean relative viability of the positive control tissues was 34.9%, which met the acceptance criterion of less than 50%. The differences in viability between identically treated tissues were 0.84% to 1.71%, which met the acceptance criterion of less than 20%. The R-squared value calculated for the plate reader linearity check was 0.9998, which met the acceptance criterion of greater than 0.999. All controls passed the acceptance criteria for a valid study.

Certificate of Analysis

SIGMA-ALDRICH

Product Name	Water, sterile-filtered, BioReagent, suitable for cell culture
Product Number	W3500
Product Brand	SIGMA
CAS Number	7732-18-5
Molecular Formula	H_2O
Molecular Weight	18.02

TEST	SPECIFICATION	LOT RNBJ0041 RESULTS
Storage:		ROOM TEMPERATURE
Print Date:		20 NOV 2019
Expiry date:		NOV 2021
Date of QC Release:		20 NOV 2019
Place of Manufacture:		Irvine. United Kingdom
Production Date:		NOV 2019
Appearance (Turbidity)	Clear	Clear
Appearance (Form)	Liquid	Liquid
pH	5.0 - 7.0	5.9
Sterility	Pass	Pass
Endotoxin Level	<= 1 EU/ml	< 1 EU/ml
Cell Culture Testing - MTT	Pass	Pass
Cell Line	Cell Line - Cell Types	Vero

MatTek Corporation

EpiOcular QC (OCL-200)

LOT 31733 TESTING DATE 4/8/2020
TESTED **Post Refrigerated Storage**
COMMENTS NO

Dosed with: 0.3% Triton X-100 (100uL)

Exposure Time (min)	Well	OD	MTT (OD)	Std Dev (OD)	Viability %	Std Dev (%)	
5	A4	1 453	1.560	0.151	90.6	8.8	Possible ET-50
	A5	1.667					
20	A6	0 96	0.943	0.024	54.8	1.4	24.05
	A7	0.926					
60	A8	0 234	0.220	0.020	12.8	1.1	22.65
	A9	0.206					
H20	A1	1 814	1.722	0.082	100.0	4.7	
	A2	1.694					
	A3	1 658					
		Avg. cv (%):	6.5	6.2	Exp. Cv (%):	6.4	

ET-50 (min): 22.65

EPIOCULAR (OCL-200) Acceptance Criteria
Based on 1996 QC Database

	TRI (MIN)	*H2O MTT (OD)*
greater than	12.2	1.10
less than	37.5	n.a.
1996 avg.	24.9	
std. dev.	6.3	

QC Evaluation: PASS

Initials: [redacted]
Date: 4/8/2020

MB Research Labs
Experience and Innovation

EpiOcular™ Eye Irritation Test (EIT)

Standard Protocol

MB Protocol Number: 722

	MB Research Standard Protocol	Page 2 of 12
Study Title:	EpiOcular™ Eye Irritation Test (EIT)	Protocol ID: 722

Section 1. OBJECTIVE	
Objective:	The purpose of this study is to provide classification of chemicals concerning their eye irritation potential using an alternative to the Draize Rabbit Eye Test, according to the OECD Test Guideline No. 492, "Reconstructed Human Cornea-like Epithelium (RhCE) Test Method for Identifying Chemicals Not Requiring Classification and Labelling for Eye Irritation or Serious Eye Damage". The EpiOcular™ EIT is intended to differentiate those materials that are UN GHS No Category (i.e., do not meet the requirements for UN GHS classification) from those that would require labeling as either UN GHS Category 1 or 2.
Limitation:	This assay is not intended to differentiate between UN GHS Category 1 and UN GHS Category 2 (nor between EU R36 and R41).

Section 2. TEST ARTICLE	
Source:	All test articles will be supplied by the Sponsor. Prior to initiation of the study, the Sponsor should provide the Study Director with test article characterization.
Characterization:	Characterization of the test article is required in support of data submissions and should include identity, strength, purity, composition, stability and uniformity. These data must be reviewed by the Study Director prior to study initiation and included in the final report. (EPA 40 CFR 160.105 and 792.105; FDA 21 CFR 58.105, OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, No.1, Sect. 6.2.) When applicable, the lack of complete, or incomplete, test article characterization will be addressed in the GLP compliance section of the report.
Label:	Each test article will be identified by source, name and/or code number, date of receipt at MB Research, and MB Project Number.
Test Article Description:	The observable physical properties of the test article will be recorded.
Safety Data Sheet:	If available, an SDS for each test article will be supplied by the Sponsor.
Storage:	Refer to *Sponsor Request* Section.
Safety:	Based on the information provided by the Sponsor, appropriate routine safety precautions will be exercised in the handling of the test article.
Analysis:	Analysis of test article in carriers (vehicles) for homogeneity and stability will not be performed unless requested by the Sponsor (at an additional cost). When applicable, the lack of analysis will be addressed in the GLP compliance statement in the final report.

Section 3. TEST SYSTEM	
Test System:	MatTek EpiOcular™ Tissue Model OCL-200 (or equivalent)
Justification:	The EpiOcular™ Tissue Model closely parallels human ocular tissue, thus providing a useful *in vitro* means to assess ocular irritancy and toxicology.
Storage:	EpiOcular™ tissues and assay medium will be refrigerated at approximately 2-8°C upon arrival and until use.

Section 4.	EXPERIMENTAL DESIGN
Plate Reader Linearity Check:	The linearity of the plate reader or spectrophotometer used for optical density (OD) determination will be verified prior to its use the same week the EIT assay is being performed. A dilution series of Trypan Blue or Thiazolyl Blue Tetrazolium Bromide (MTT) formazan will be prepared and 200 µl aliquots will be pipetted into a 96-well plate. The optical density of the plate wells will be measured at a wavelength of 570 nm (OD_{570}), with no reference wavelength. A regression line and an R-squared value will be generated using Microsoft Excel®. Verification will be considered acceptable if the R-squared value is >0.999.
Reduction of MTT:	Direct MTT reduction potential of the test article will be assessed prior to starting the main assay. A 1 mg/ml MTT solution, diluted in Dulbecco's Modified Eagle's Medium (DMEM) will be prepared and protected from light. A 50 µl or 50 mg aliquot of the test article will be added to 1 ml of the MTT solution in a single well of a six-well plate. A negative control, 50 µl of tissue culture water, will be tested concurrently. The mixtures will be incubated in the dark at 37±1°C and 5±1% CO_2 for approximately 3 hours. If the test article results in a reduction of MTT (by visual assessment), then four frozen (dead) tissues will be assayed concurrently in the main assay. Two dead tissues will be treated with tissue culture water as controls to ensure that the tissues are dead. The other two tissues will be treated with test article (See Experimental Design, Killed MTT Controls).

Section 4.	EXPERIMENTAL DESIGN (cont'd)
Assessment of Coloring or Staining Materials:	Colored test articles must be assessed for their ability to absorb light at the wavelength used for MTT determination (570 nm). • 50 µl (liquid) or 50 mg (solid) of the test article will be incubated for 2 to 3 hours in a six-well plate, with 2 ml of extractant (isopropanol) at room temperature with shaking. • If the OD_{570} of the extractant is >0.08 after subtraction of blank (undosed isopropanol), then colorant controls must be performed on two additional live tissues (See Experimental Design, Colorant Controls). Non-colored test articles must be assessed to determine if they will become colored in water or extractant. • 50 µl (liquid) or 50 mg (solid) of the test article will be incubated in a six-well plate with 1 ml of tissue culture water for at least one hour in a humidified 37±1°C, 5±1% CO_2 incubator. • Also, 50 µl (liquid) or 50 mg (solid) of the test article will be incubated in a six-well plate with 2 ml extractant at room temperature for 2 to 3 hours. • If color develops in either the water or extractant, resulting in an OD_{570} >0.08 after subtraction of blank (undosed tissue culture water or isopropanol, respectively), then colorant controls must be performed using two additional live tissues (See Experimental Design, Colorant Controls).
Test articles that are both MTT Reducers and Colorants:	Require Killed MTT Controls, Colorant Controls AND an additional set of two dead tissues. (See Experimental Design, Killed Controls for Test Articles that are both MTT Reducers and Colorants).
Test Article Exposure Times:	**Liquid test articles:** Two tissues per test article will be exposed for 30 ± 2 minutes. **Solid test articles:** Two tissues per test article will be exposed for 6 hours ± 15 minutes.
Negative Controls:	Two tissues will be dosed with 50 µl of tissue culture water and treated in the same manner as tissues treated with test article(s). For liquid test articles, the negative controls will be exposed for 30 ± 2 minutes; for solid test articles, the negative controls will be exposed for 6 hours ± 15 minutes. If both liquids and solids are being tested with the same lot of tissues, two sets of negative controls will be necessary.
Positive Controls:	Two tissues will be dosed will 50 µl methyl acetate and treated in the same manner as tissues treated with test article(s). For liquid test articles, the positive controls will be exposed for 30 ± 2 minutes; for solid test articles, the positive controls will be exposed for 6 hours ± 15 minutes. If both liquids and solids are being tested with the same lot of tissues, two sets of positive controls will be necessary.

Section 4. EXPERIMENTAL DESIGN (cont'd)	
Killed MTT Controls:	When used (see Table 1), these controls will be treated in the same manner as the test tissues.
Colorant Controls:	When used (see Table 1), these controls will be treated in the same manner as the test tissues, except they will be incubated in assay media instead of MTT during the MTT incubation period (see Section 6).
Killed Controls for Test Articles that are both MTT Reducers and Colorants:	When used (see Table 1), these controls will be treated in the same manner as the test tissues, except they will be incubated in assay media during the MTT incubation period (see Section 6).

TABLE 1

	MTT Reduction Controls	Colorant Controls	MTT Reduction / Colorant Killed Controls
Needed if:	Test article directly reduces MTT (See Section 5: Reduction of MTT)	Test article OD_{570} >0.08 when incubated in extractant or water (See Section 5: Assessment of Coloring or Staining Materials)	Test article directly reduces MTT and OD_{570} >0.08
Additional Tissues:	4 dead tissues • 2 treated with tissue culture water • 2 treated with test article • incubated in MTT during MTT incubation (same as live test tissues)	2 live tissues • treated with test article • incubated in assay medium instead of MTT during the MTT incubation period	8 total additional tissues • 2 dead tissues treated with test article, incubated in assay medium PLUS • **MTT Reduction Controls** (4 dead tissues) • **Colorant Controls** (2 live tissues)

Section 5.	TEST PROCEDURES
Pre-Incubation:	EpiOcular™ tissues will be placed in six-well plates containing warmed assay media and will be equilibrated in a humidified 37±1°C, 5 ±1% CO_2 incubator for at least one hour. The media will then be changed and the tissues incubated overnight (16-24 hours). Any tissues not being incubated the same day will be allowed to re-equilibrate at 37±1°C, 5±1% CO_2 and will be stored at approximately 2-8°C.
Pre-Treatment:	After the overnight incubation, the tissues will be moistened with 20 µl of Phosphate-Buffered Saline (PBS) and incubated at 37 ±1°C, 5±1% CO_2 for 30 ± 2 minutes.
Dosing:	**Liquids:** 50 µl of a liquid test article will be applied topically to duplicate tissues and incubated at 37±1°C, 5±1% CO_2 for 30 ± 2 minutes. **Viscous, waxy, resinous and gel-like** test articles with unclear physical state should be incubated at 37±1°C for 15±1 minutes before deciding which treatment protocol to use. If such test articles become pipettable after this incubation period (using a positive displacement pipette, if necessary), they should be treated as liquids and should be applied to the tissues directly from the water bath (at 37±1°C), otherwise they should be treated as solids. Applicator pins inverted onto the tissue may be used to provide a reproducible, even means of application. **Solids:** Whenever possible, solids should be ground to a fine powder before application. 50 mg of a solid test article will be applied topically to duplicate tissues and incubated at 37±1°C, 5±1% CO_2 for 6 hours ± 15 minutes.
Rinse/Soak:	After dosing and incubation, the tissues will be thoroughly rinsed with PBS and soaked in 5 ml of room-temperature assay medium in a 12-well plate for the appropriate amount of time. **Liquids:** Tissues will be soaked for 12 ± 2 minutes. **Solids:** Tissues will be soaked for 25 ± 2 minutes.
Post-Treatment Incubation:	Tissues will be incubated in 1 ml fresh assay medium in a humidified 37±1°C, 5±1% CO_2 incubator. **Liquids** will be incubated for 120 ± 15 minutes. **Solids** will be incubated for 18 hours ± 15 minutes.
Incubation in MTT:	The tissues will be incubated with 300 µl of 1 mg/ml MTT in DMEM for 3 hours ± 10 minutes at 37±1°C, 5±1% CO_2 • Tissues for Colorant Controls and Killed Controls for Test articles that are both MTT Reducers and Colorants will be incubated in assay media instead of MTT during the MTT incubation period.

	MB Research Standard Protocol	Page 7 of 12
Study Title:	EpiOcular™ Eye Irritation Test (EIT)	Protocol ID: 722

Section 5.	TEST PROCEDURES (cont'd)
MTT Extraction:	Following the three-hour MTT incubation period, each tissue will be removed individually and gently rinsed with PBS to remove any residual MTT solution. For liquid test articles, the tissues will be immersed in 2.0 ml of extractant solution per well in a 24-well plate, completely covering the EpiOcular™ tissue (i.e., extraction will occur through both the top and bottom of the insert). For solid, colored, or staining test articles, 2.0 ml of extractant solution will be used in a six-well plate, allowing extraction to occur through the bottom of the insert. The extraction plate will be covered and sealed to reduce evaporation of extractant.
Extraction Conditions:	The extraction will be allowed to proceed overnight at room temperature in the dark. Alternatively, the extraction can proceed for at least two hours, with shaking, at room temperature.
Decant Extractant:	Tissues immersed in extractant solution in a 24-well plate: After the extraction period is complete, the liquid within each tissue insert will be decanted back into the well from which it was taken, i.e., the solution will be mixed with the extractant in the well. Tissues extracted in six-well plate: No liquid is decanted from the tissue insert. The tissue inserts will then be discarded.
Transferring to 96-Well Plate:	Two 200 µl aliquots from each well of the extraction plate(s) will be pipetted into a 96-well microtiter plate. Note: If a 96-well plate reader is not available, any visible spectrophotometer will be used to determine optical density of the extracted samples.
Measuring Optical Density:	The optical density of the extracted samples will be determined at a single wavelength of 570 nm and using eight 200 µl aliquots of the Extractant as blanks.

	MB Research Standard Protocol	Page 8 of 12
Study Title:	EpiOcular™ Eye Irritation Test (EIT)	Protocol ID: 722

Section 6. DATA ANALYSIS	
Calculating Percent Viability:	The percent viability of the test tissues will be determined using the following formula: % Viability = 100 x (OD_{sample} / $OD_{Negative\ Control}$)
Killed MTT Controls:	If the resulting calculated viability is less than 50%, the calculated mean viability of the test tissues will be corrected for MTT reduction caused by the test article. (See Table 2) Corrected Viability = % Viability –Mean Apparent Viability$_{Test\ Article\text{-}treated\ killed\ controls\ incubated\ in\ MTT}$
Colorant Controls:	If the resulting calculated viability is less than 50%, the calculated mean viability of the test tissues will be corrected for color caused by the test article. (See Table 2) Corrected Viability = % Viability – Mean Apparent Viability$_{Test\ Article\text{-}treated\ colorant\ (live)\ controls}$
Killed Controls for Test articles that are both MTT Reducers and Colorants:	The resulting calculated mean viability of these killed controls will be ADDED to the calculated mean viability of the test tissues after the mean killed MTT control viability and mean colorant control viability have been subtracted. (See Table 2) Corrected Viability = % Viability – V_A – V_B + V_C Where: V_A = Mean Apparent Viability of the Test Article-treated killed controls incubated in MTT V_B = Mean Apparent Viability of the Test Article-treated colorant (live) controls V_C = Mean Apparent Viability of the Test Article-treated killed controls incubated in assay medium

TABLE 2

	MTT Reduction Controls	Colorant Controls	MTT Reduction / Colorant Killed Controls
Calculated Apparent Viability – Test Article-treated Controls:	Viability ≥ 50% • Test article is incompatible with the assay* Viability < 50% • SUBTRACT the mean apparent viability of the test article-treated killed controls from mean viability of the test article-treated live tissues to correct for direct MTT reduction.	Viability ≥ 50% • Test article is incompatible with the assay* Viability < 50% • SUBTRACT the mean apparent viability of the colorant controls from mean viability of the test article-treated test tissues to correct for color caused by the test article.	Viability ≥ 50% • Test article is incompatible with the assay* Viability < 50% • SUBTRACT the mean apparent viability of the test article-treated killed controls (incubated in MTT) from mean viability of the test article-treated live tissues to correct for direct MTT reduction. • SUBTRACT the mean apparent viability of the colorant controls (live tissues) from mean viability of the test article-treated test tissues to correct for color caused by the test article. • ADD the mean apparent viability of the test article - treated killed controls (incubated in assay medium) from mean viability of the test article-treated live tissues.
*unless the results of the viable test tissues indicate an irritating result, i.e., test tissue viability is ≤ 60% relative to mean negative control-treated tissue viability without any MTT or negative control correction			

	MB Research Standard Protocol	Page 9 of 12
Study Title:	EpiOcular™ Eye Irritation Test (EIT)	Protocol ID: 722

Section 6. DATA ANALYSIS (cont'd)	
Quality Controls:	Negative Controls: The assay meets the acceptance criterion if the OD_{570} of the Negative Control is >0.8 and <2.5.
	Positive Controls: The assay meets the acceptance criterion if the mean relative viability of the positive control is below 50% of negative control viability.
	Tissue Variability: The difference in viability between identically treated tissues must be less than 20%. This applies to tissues treated with the same test article as well as living and killed controls.
Irritation Potential:	If the mean test article-treated tissue viability is > 60% relative to mean negative control-treated tissue viability, the test article will be classified as UN GHS No Category, and is therefore interpreted to be non-irritating. If the mean test article-treated tissue viability is ≤ 60% relative to mean negative control-treated tissue viability, the test article will be considered an ocular irritant (UN GHS Category 1 or 2).
Borderline Results:	If the test article-treated tissue viability is 60±5%, a second EIT should be performed. If the results of the second test disagree with the first, then a third test should be performed. The conclusion will be based on the agreement of two of the three tests.

Section 7. TEST DURATION	
Duration:	The duration of the EpiOcular™ Eye Irritation Test is approximately five days.

Section 8. REFERENCES
1. OECD Guideline for the Testing of Chemicals, No. 792: Reconstructed Human Cornea-like Epithelium (RhCE) Test Method for Identifying Chemicals Not Requiring Classification and Labeling for Eye Irritation or Serious Eye Damage
2. MatTek Corporation Protocol: EpiOcular™ Eye Irritation Test (OCL-200-EIT)

Section 9. PROTOCOL REVISIONS	
Revisions:	Any amendment to or deviation from this protocol will be fully documented in the study file, including the reason for the change, the authority for said change and the date.

	MB Research Standard Protocol	Page 10 of 12
Study Title:	EpiOcular™ Eye Irritation Test (EIT)	Protocol ID: 722

Section 10. RECORDS TO BE MAINTAINED	
Collection of Data:	All data generated during the conduct of this study will be recorded in ink on data collection forms. All entries will be dated, initialed and verified per MB Standard Operating Procedures (SOPs).
Final Report:	The final report will include, but is not limited to, a description of the methods and experimental design, results, discussion, conclusion, data tables and the Quality Assurance statement. The content of the final report will meet the requirements of the applicable Good Laboratory Practice Regulations.
Retention of Data:	All data generated during the conduct of this study will be archived at MB Research for at least ten years from the date of the final report. The Sponsor will be contacted in writing to determine final disposition of the records. If the Sponsor fails to respond within 90 days, the archived items will be properly discarded.
Raw Data:	Raw data will be filed at MB Research by project number.
Final Reports:	The final report will be filed at MB Research by Sponsor name and MB project number.
Test Article:	Refer to the *Sponsor Request* Section for test article disposition. If this study exceeds 28 days, it is recommended that the Sponsor archive a sample of the test article to meet the applicable Good Laboratory Practices Regulations.
Test Article Mixtures:	These are not routinely retained. However, upon written request from the Sponsor, an aliquot of the test article mixture will be forwarded to the Sponsor (at an additional cost).

Section 11. GOOD LABORATORY PRACTICES	
This study will be conducted in accordance with the current Good Laboratory Practice Regulations of the EPA, 40 CFR Part 160 and 792, the FDA, 21 CFR Part 58, and OECD, Principles on Good Laboratory Practice.	
Protocol:	MB Research will have on file a copy of this protocol, signed and dated by the responsible MB Study Director and dated by the Sponsor Representative.
Quality Assurance:	The Quality Assurance Unit will inspect at least one critical phase of this study, audit the raw data and audit the report in accordance with the protocol, MB Research SOPs and applicable regulatory requirements.

	MB Research Standard Protocol	Page 11 of 12
Study Title:	EpiOcular™ Eye Irritation Test (EIT)	Protocol ID: 722

Section 12. SPONSOR REQUEST			
Protocol:	Implement as written		Use 1 min, 4 min, and 16 min times
Report Submission:	No, this report will not be submitted		
Test Article Identity:	*(The identity that will be used in the **report** and supporting documentation):* [redacted] F # 72AO-88		
Characterization:	No, test article characterization is not provided.	*(if provided)*	
Storage Requirements:	Room temperature	No protection from light is necessary	Test article is NOT oxygen-sensitive
Dosing:	Neat	*(Vehicle, if diluted):*	*(Concentration, if diluted):*
Additional Information:			
Disposition:	Discard at study termination - no charge		
Authorization Statement:	This protocol is authorized for implementation at MB Research.		
Confidentiality:	Study results and reports will be released only to the below-named Sponsor representative unless other Sponsor representatives are identified below.		
Authorization Date:	05Mar2020		
Sponsor Name:	[redacted]	**Title:** R&D Coordinator	
Email Address:	[redacted]		
Company Name:	[redacted]		
Additional Sponsor Representative(s):	[redacted]		

	MB Research Standard Protocol	Page 12 of 12
Study Title:	EpiOcular™ Eye Irritation Test (EIT)	Protocol ID: 722

Section 13. MB RESEARCH ACKNOWLEDGEMENT			
Request for implementation of this protocol and receipt of the test article is acknowledged by MB Research.			
Test Article Identity:	[redacted] F # 72AO-88		
Characterization:[1]	No, test article characterization was not provided.		
MB Project #:	20-27939.19		
Tissue Supplier:	The tissue equivalent supplier is: MatTek		
Proposed Experimental Start Date:	06 Apr 2020	**Proposed Experimental Term Date:**	10 Apr 2020
Completion Date:	The report will be submitted approximately four weeks following the experimental term date.		
Approval:	This protocol is approved for implementation at MB Research by the below-named MB Study Director.		
Approved By:	[redacted] Date: 03 Apr 2020 Time: 1620		
[redacted] Testing Facility: MB Research Laboratories 1765 Wentz Road, P. O. Box 178 Spinnerstown, PA 18968			

[1] Page revised 01 Dec 2016 to clarify provision of test article characterization

BioScreen® Testing Services, Inc.
3892 Del Amo Boulevard • Torrance, California 90503
(310) 214-0043
Web Site: www.bioscreen.com • E-Mail: info@bioscreen.com

100 SUBJECT HUMAN REPEAT INSULT PATCH TEST FOR SKIN IRRITATION AND SKIN SENSITIZATION EVALUATION

Date: September 15, 2021

BCS Study No.: 21-527A & 21-528A

Sponsor: [redacted]

1.0 Objective: To determine the irritation and sensitization (contact allergy) potential of a test material after repeated application to the skin of human subjects.

2.0 Test Material:

2.1 Test Material Description:

The use concentration for IPC in this study is 0.0075%.

Date Received: May 26, 2021

Received From: [redacted]

Number of Test Samples Received: 1

Label on Test Samples: [redacted]
Formula Number: 66IM-16,
Lot/Batch# N/A

Accession No.: 1165900

2.2 Handling:

Upon arrival at BioScreen Clinical Services (BCS) the test material was assigned a unique laboratory code number and entered into a daily log identifying the lot number, sample description, sponsor, date received and tests requested.

Samples will be retained for a period of thirty (30) days

beyond submission of final report unless otherwise specified by the sponsor or, if sample is known to be in support of governmental applications, in which case representative retained samples are kept two (2) years beyond final report submission.

Sample disposition will be conducted in compliance with appropriate federal, state and local ordinances.

3.0 Panel Selection:

3.1 Standards for Inclusion in a Study:

- Individuals who were not currently under a doctor's care.
- Individuals who were free of any dermatological or systemic disorder that would interfere with the results, at the discretion of the Investigator.
- Individuals who were free of any acute or chronic disease that would interfere with or increase the risk of study participation.
- Individuals who completed a preliminary medical history form mandated by BCS and were in general good health.
- Individuals who read, understood and signed an informed consent document relating to the specific type of study.
- Individuals who were able to cooperate with the Investigator and research staff, and were willing to have test materials applied according to the protocol, and complete the full course of the study.

3.2 Standards for Exclusion from a Study:

- Individuals who were under 18 years of age.
- Individuals who were currently under a doctor's care.
- Individuals who were currently taking any medication (topical or systemic) that might mask or interfere with the test results.
- Individuals who had a history of any acute or chronic disease that might interfere with or increase the risk associated with study participation.
- Individuals who were diagnosed with chronic skin allergies.
- Female volunteers who indicated that they were pregnant or nursing.

3.3 Recruitment:

Panel selection was accomplished by advertisements in local periodicals, community bulletin boards, phone solicitation, electronic media or any combination thereof.

3.4 Informed Consent and Medical History Forms:

An informed consent was obtained from each volunteer prior to initiating the study describing reasons for the study, possible adverse effects, associated risks and potential benefits of the treatment and their limits of liability. Panelists signed and dated the informed consent document to indicate their authorization to proceed and acknowledge their understanding of the contents. Each subject was assigned a permanent identification number and completed an extensive medical history form. These forms along with the signed consent forms are available for inspection on the premises of BCS only. [Reference 21 CFR Ch. 1 Part 50, Subpart B]

The parties agree to comply with applicable state and federal privacy laws for the use and disclosure of a subject's personal health information by taking reasonable steps to protect the confidentiality of this information. This obligation shall survive the termination or expiration of this Agreement.

4.0 Population Demographics:

Number of subjects enrolled	110
Number of subjects completing study	102
Age Range	18-64
Sex	
Male	23
Female	79
Fitzpatrick Skin Type*	
1 – always burn, does not tan	0
2 – burn easily, tan slightly	11
3 – burn moderately, tan progressively	54
4 – burn a little, always tan	36
5 – rarely burn, tan intensely	1
6 – never burn, tan very intensely	0

*[Agache P., Hubert P.. Measuring the skin. (p. 473, table 48.1) Springer-Verlag Berlin Heidelberg, 2004, (p. 473, table 48.1)

5.0 Equipment:

Test materials to be tested under occlusive conditions were placed on an 8-millimeter aluminum Finn Chamber® (Epitest Ltd. Oy, Tuusula, Finland) supported on Scanpor® Tape (Norgesplaster A/S, Kristiansand, Norway) or an 8-millimeter filter paper coated aluminum Finn Chamber® AQUA supported on a thin flexible transparent polyurethane rectangular film coated on one side with a medical grade acrylic adhesive, consistent with adhesive used in state-of-the-art hypoallergenic surgical tapes or a 7mm IQ-ULTRA® closed cell system which is made of additive-free polyethylene plastic foam with a filter paper incorporated (It is supplied in units of 10 chambers on a hypoallergenic non woven adhesive tape; the width of the tape is 52mm and the length is 118mm) or other equivalents.

Test materials to be tested under semi-occlusive conditions were placed on a test strip with a Rayon/Polypropylene pad or on a 7.5mm filter paper disc affixed to a strip of hypoallergenic tape (Johnson & Johnson 1 inch First Aid Cloth Tape).

Test materials to be tested in an open patch were applied and rubbed directly onto the back of the subject.

Approximately 0.02-0.05 mL (in case of liquids) and/or 0.02-0.05 gm (in case of solids) of the test material was used for the study. Liquid test material was dispensed on a 7.5mm paper disk, which fit in the Finn Chamber.

6.0 Procedure:

- Subjects were requested to bathe or wash as usual before arrival at the facility.
- Patches containing the test material were then affixed directly to the skin of the intrascapular regions of the back, to the right or left of the midline and subjects were dismissed with instructions not to wet or expose the test area to direct sunlight.
- Patches remained in place for 48 hours after the first application. Subjects were instructed not to remove the patches prior to their 48 hour scheduled visit. Thereafter, subjects were instructed to remove patches 24 hours after application for the remainder of the study.

- This procedure was repeated until a series of nine (9) consecutive, 24-hour exposures had been made three (3) times a week for three (3) consecutive weeks.
- Prior to each reapplication, the test sites evaluated by trained laboratory personnel.
- Following a 10-14 day rest period a retest/challenge dose was applied once to a previously unexposed test site. Test sites were evaluated by trained laboratory personnel 48 and 96 hours after application.
- In the event of an adverse reaction, the area of erythema and edema were measured. Edema is estimated by the evaluation of the skin with respect to the contour of the unaffected normal skin.
- Subjects were instructed to report any delayed reactions that might occur after the final reading.
- Clients will be notified immediately in the case of an adverse reaction and a determination is made as to treatment program if necessary.

7.0 Scoring:

Scoring scale and definition of symbols shown below are based on the scoring scheme according to the International Contact Dermatitis Research Group scoring scale [Rietschel, R.L., Fowler, J.F., Ed., Fisher's Contact Dermatitis (fourth ed.). Baltimore, Williams & Wilkins, 1995] listed below:

0 no reaction (negative)
1 erythema throughout at least ¾ of patch area
2 erythema and induration throughout at least ¾ of patch area
3 erythema, induration and vesicles
4 erythema, induration and bullae

D Site discontinued
Dc Subject discontinued voluntarily
DcI Subject discontinued per Investigator

NOTE: Clinical evaluations are performed by a BCS investigator or designee trained in the clinical evaluation of the skin. Whenever feasible, the same individual will do the scoring of all the subjects throughout the study and will be blinded to the treatment assignments and any previous scores.

8.0 Results:

Accession No.: 1165900
Test Material Description: [redacted] Formula Number:
66IM-16, Lot/Batch# N/A
Patch Description: Occlusive

Subject Information					Induction									Challenge	
No.	Subject ID	Sex	Age	Skin Type	1	2	3	4	5	6	7	8	9	1	2
1	3000028	F	63	3	0	0	0	0	0	0	0	0	0	0	0
2	3000044	M	60	3	0	0	0	0	0	0	1	0	0	0	0
3	3000073	F	41	2	0	0	0	0	0	0	0	0	0	0	0
4	3000074	F	51	3	0	0	0	0	0	0	1	0	0	0	0
5	3000142	F	41	4	0	0	0	0	0	0	0	0	0	0	0
6	3000196	F	39	4	0	0	0	0	0	0	0	0	0	0	0
7	3000282	M	45	4	0	0	0	0	0	0	0	0	0	0	0
8	3000292	F	41	4	0	0	0	0	0	0	1	0	0	1	0
9	3000320	F	56	3	0	0	0	0	0	0	0	0	0	0	0
10	3000328	F	24	2	0	0	0	0	0	0	0	0	0	0	0
11	3000341	M	48	4	0	0	0	0	0	0	0	0	0	0	0
12	3000373	F	24	3	0	0	0	0	0	0	0	0	0	0	0
13	3000376	F	47	3	0	0	0	0	0	0	0	0	0	0	0
14	3000821	F	37	3	0	0	0	0	0	0	0	0	0	0	0
15	3000825	F	54	4	0	0	0	0	0	0	0	0	0	0	0
16	3000835	F	56	3	0	0	0	0	0	0	0	0	0	0	0
17	3000906	F	35	3	0	0	0	0	0	0	0	0	0	0	0
18	3001956	F	42	3	0	0	0	0	0	0	0	0	0	0	0
19	3002034	M	64	3	0	0	0	0	0	0	0	0	0	0	0
20	3002036	F	64	3	0	0	0	0	0	0	0	0	0	0	0
21	3002069	F	46	4	0	0	0	0	0	0	0	0	0	0	0
22	3002078	F	43	3	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc
23	3003200	F	47	3	0	0	0	0	0	0	0	0	0	0	0
24	3005204	F	42	3	0	0	0	0	0	0	0	0	0	0	0
25	3005218	F	47	3	0	0	0	0	0	0	0	0	0	0	0
26	3005249	F	38	4	0	0	0	0	0	0	0	0	0	0	0
27	3005256	F	23	3	0	0	0	0	0	0	0	0	0	0	0
28	3005257	F	60	4	0	0	0	0	0	0	0	0	0	0	0
29	3007289	F	59	3	0	0	0	0	0	0	0	0	0	0	0
30	3007311	F	22	3	0	0	0	0	0	0	0	0	0	0	0
31	3009324	F	49	3	0	0	0	0	0	0	0	0	0	0	0
32	3009329	F	31	3	0	0	0	0	0	0	0	0	0	0	0
33	3009396	F	63	3	0	0	0	0	0	0	0	0	0	0	0

34	3009397	F	48	3	0	0	0	0	0	0	0	0	0	0	0
35	3010550	F	39	3	0	0	0	0	1	0	0	0	0	0	0
36	3010570	F	48	2	0	0	0	0	0	0	0	0	0	0	0
37	3010586	F	55	4	0	0	0	0	0	0	0	0	0	0	0
38	3010587	F	61	3	0	0	0	0	0	0	0	0	0	0	0
39	3010620	F	40	3	0	0	0	0	0	0	0	0	0	0	0
40	3010624	F	55	3	0	0	0	0	1	0	0	0	0	0	0
41	3010656	F	42	2	0	0	0	0	0	0	0	0	0	0	0
42	3010733	F	38	3	0	0	0	0	0	0	0	0	0	0	0
43	3010751	F	41	3	0	0	1	0	0	0	0	0	0	0	0
44	3010763	M	21	2	0	0	0	0	0	0	0	0	0	0	0
45	3010771	F	31	3	0	0	0	0	0	0	0	0	0	0	0
46	3010794	M	43	2	0	0	0	0	0	0	0	0	0	0	0
47	3018015	F	48	4	0	0	0	0	0	0	0	0	0	0	0
48	3018059	M	45	3	0	0	0	0	0	0	0	0	0	0	0
49	3018061	F	59	4	0	0	0	0	0	0	0	0	0	0	0
50	3020231	F	35	3	0	0	0	0	0	0	0	0	0	0	0
51	3021206	F	25	3	0	0	0	0	0	0	0	0	0	0	0
52	3021352	M	44	3	0	0	0	0	0	0	0	0	0	0	0
53	3021366	F	42	4	0	0	0	0	0	0	0	0	0	0	0
54	3022393	F	51	4	0	0	0	0	0	0	0	0	0	0	0
55	3022428	F	54	3	0	0	0	0	0	0	0	0	0	0	0
56	3022441*	F	30	3	0	0	0	0	Dcl	Dcl	Dcl	Dcl	Dcl	Dcl	Dcl
57	3022442	F	35	4	0	0	0	0	0	0	0	0	0	0	0
58	3022443	F	19	3	0	0	0	0	0	0	0	0	0	0	0
59	3022456	F	51	4	0	0	0	0	0	0	0	0	0	0	0
60	3022656	M	25	2	0	0	0	0	0	0	0	0	0	0	0
61	3022671	F	33	2	0	0	0	0	0	0	0	0	0	0	0
62	3022679	F	24	3	0	0	0	0	0	0	0	0	0	0	0
63	3022705	F	50	4	0	0	0	0	0	0	0	0	0	0	0
64	3022711	M	61	2	0	0	0	0	0	0	0	0	0	0	0
65	3022733	F	24	4	0	0	0	0	0	0	0	0	0	0	0
66	3022737	F	21	4	0	0	0	0	0	0	0	0	0	0	0
67	3022812	F	20	4	0	0	0	0	0	0	0	0	0	0	0
68	3022821	F	32	4	0	0	0	0	0	0	0	0	0	Dc	Dc
69	3023035	M	23	3	0	0	0	0	0	0	0	0	0	0	0
70	3023071	F	31	3	0	0	0	0	0	0	0	0	0	0	0
71	3023106	F	45	3	0	0	0	0	0	0	0	0	0	0	0
72	3023107	M	18	3	0	0	0	0	0	0	0	0	0	0	0
73	3023167	M	27	4	0	0	0	0	0	0	0	0	0	0	0
74	3023205	F	42	3	0	0	0	0	0	0	0	0	0	0	0

75	3023226	F	45	4	0	0	0	0	0	0	0	0	0	0	0
76	3023237	F	32	4	0	0	0	0	0	0	0	0	0	0	0
77	3023258	M	39	3	0	0	0	0	0	0	0	0	0	0	0
78	3023259	F	33	3	0	0	0	0	0	0	0	0	0	0	0
79	3023261	F	55	5	0	0	0	0	0	1	0	0	0	0	0
80	3023265	F	22	3	0	0	0	0	0	0	0	0	0	0	0
81	3023268	F	50	3	0	0	0	0	0	0	0	0	0	0	0
82	3023273	M	20	3	0	0	0	0	0	0	0	0	0	0	0
83	3023582	F	22	4	0	0	0	0	0	0	0	0	0	Dc	Dc
84	3023583	F	19	4	0	0	0	0	0	0	0	0	0	0	0
85	3023584	F	33	4	0	0	0	0	0	0	0	0	0	0	0
86	3023585	M	35	2	0	0	0	0	0	0	0	0	0	0	0
87	3023586	F	25	3	0	0	0	0	0	0	0	0	0	0	0
88	3023587	M	35	4	0	0	0	0	0	0	0	0	0	0	0
89	3023588	M	18	4	0	0	0	0	0	0	0	0	0	0	0
90	3023589	F	19	2	0	0	0	0	0	0	0	0	0	0	0
91	3023590	M	21	5	0	0	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc
92	3023591	M	27	4	0	0	0	0	0	0	0	0	0	0	0
93	3023592	M	19	3	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc
94	3023593	M	23	3	0	0	0	0	0	0	0	0	0	0	0
95	3023595	F	18	3	0	0	0	0	0	0	0	0	0	0	0
96	3023596	F	32	4	0	0	0	0	0	0	0	0	0	0	0
97	3023598	M	34	3	0	0	0	0	0	0	0	0	0	0	0
98	3023600	F	58	3	0	0	0	0	0	0	0	0	0	0	0
99	3023601	F	30	4	0	0	0	0	0	0	0	0	0	0	0
100	3023602	F	29	4	0	0	0	0	0	0	0	0	0	0	0
101	3023603	F	28	4	0	0	0	0	0	0	0	0	0	0	0
102	3023604	F	35	3	0	0	0	0	0	0	0	0	0	0	0
103	3023605*	F	36	4	0	Dcl	Dcl	Dcl	Dcl	Dcl	Dcl	Dcl	Dcl	Dcl	Dcl
104	3023606	M	57	3	0	0	0	0	0	0	0	0	0	0	0
105	3023607	F	43	4	0	0	0	0	0	0	0	0	0	0	0
106	3023608	M	18	4	0	0	0	0	0	0	0	0	0	Dc	Dc
107	3023609	F	41	4	0	0	0	0	0	0	1	0	0	0	0
108	3023610	F	19	4	0	0	0	0	0	0	0	0	0	0	0
109	3023611	M	35	4	0	0	0	0	0	0	0	0	0	0	0
110	3023612	F	18	3	0	0	0	0	0	0	0	0	0	0	0

*Drop due to study criteria.

9.0 Evaluation Period:

The study was conducted from June 28, 2021 to, August 6 2021.

10.0 Observations:

No adverse reactions of any kind were reported during the course of this study.

There were one (1) subject with a Grade 4 reaction, six (6) subjects with a Grade 2 reaction, and sixty-five (65) subjects with a Grade 1 reaction to the positive control (2.0% Sodium Lauryl Sulfate Solution).

No subjects showed any signs of reaction to the negative control (DI Water).

11.0 Study Archives:

All original samples, raw data sheets, technician's notebooks, correspondence files and copies of final reports and remaining specimens will be maintained on premises of BCS in limited access storage files marked "Archive".

12.0 Conclusions:

Under the conditions of the study, there was no indication of a potential to elicit dermal irritation noted for [redacted] Formula Number: 66IM-16, Lot/Batch# N/A; Accession No. 1165900.

There was one (1) Grade 1 reaction observed at Challenge 1 for Subject 3000292. Re-challenge was performed.

1 SUBJECT RE-CHALLENGE FOR SKIN SENSITIZATION EVALUATION

1.0 Objective: To determine the sensitization potential of a test product where previous reactions were observed during the challenge phase of a Human Repeat Insult Patch Test (HRIPT).

2.0 Test Material:

2.1 Re-challenge Test material Description:

Date Received: May 26, 2021

Received From: [redacted]

Number Of Test Samples Received: 1

Label On Test Samples: [redacted]
Formula Number: 66IM-16,
Lot/Batch# N/A

Accession No.: 1165900

2.2 Handling:

Upon arrival at BioScreen Clinical Services (BCS) the test material was assigned a unique laboratory code number and entered into a daily log identifying the lot number, sample description, sponsor, date received and tests requested.

Samples will be retained for a period of thirty (30) days beyond submission of final report unless otherwise specified by the sponsor or, if sample is known to be in support of governmental applications, in which case representative retained samples are kept two (2) years beyond final report submission.

Sample disposition will be conducted in compliance with appropriate federal, state and local ordinances.

3.0 Panel Selection:

3.1 Standards for Inclusion in a Study:

- Individuals whom showed an irritation reaction during challenge phase of an HRIPT.

3.2 Standards for Exclusion from a Study:

- Individuals who were under 18 years of age.
- Individuals who were currently under a doctor's care.
- Individuals who were currently taking any medication (topical or systemic) that might mask or interfere with the test results.
- Individuals who had a history of any acute or chronic disease that might interfere with or increase the risk associated with study participation.
- Individuals who were diagnosed with chronic skin allergies.
- Female volunteers who indicated that they were pregnant or nursing.

3.3 Recruitment:

Panel selection was accomplished by advertisements in local periodicals, community bulletin boards, phone solicitation, electronic media or any combination thereof.

3.4 Informed Consent and Medical History Forms:

An informed consent was obtained from each volunteer prior to initiating the study describing reasons for the study, possible adverse effects, associated risks and potential benefits of the treatment and their limits of liability. Panelists signed and dated the informed consent document to indicate their authorization to proceed and acknowledge their understanding of the contents. Each subject was assigned a permanent identification number and completed an extensive medical history form. These forms along with the signed consent forms are available for inspection on the premises of BCS only. [Reference 21 CFR Ch. 1 Part 50, Subpart B]

The parties agree to comply with applicable state and federal privacy laws for the use and disclosure of a subject's personal health information by taking reasonable steps to protect the confidentiality of this information. This obligation shall survive the termination or expiration of this Agreement.

4.0 Population Demographics:

Number of subjects enrolled	1
Number of subjects completing study	1

Age Range	41
Sex	
Male	0
Female	1
Fitzpatrick Skin Type*	
1 – always burn, does not tan	0
2 – burn easily, tan slightly	0
3 – burn moderately, tan progressively	0
4 – burn a little, always tan	1
5 – rarely burn, tan intensely	0
6 – never burn, tan very intensely	0

*[Agache P., Hubert P.. Measuring the skin. (p. 473, table 48.1) Springer-Verlag Berlin Heidelberg, 2004, (p. 473, table 48.1)]

5.0 Equipment:

Test materials to be tested under occlusive conditions were placed on an 8-millimeter aluminum Finn Chamber® (Epitest Ltd. Oy, Tuusula, Finland) supported on Scanpor® Tape (Norgesplaster A/S, Kristiansand, Norway) or an 8-millimeter filter paper coated aluminum Finn Chamber® AQUA supported on a thin flexible transparent polyurethane rectangular film coated on one side with a medical grade acrylic adhesive, consistent with adhesive used in state-of-the-art hypoallergenic surgical tapes or a 7mm IQ-ULTRA® closed cell system which is made of additive-free polyethylene plastic foam with a filter paper incorporated (It is supplied in units of 10 chambers on a hypoallergenic non woven adhesive tape; the width of the tape is 52mm and the length is 118mm) or other equivalents.

Approximately 0.02-0.05 mL (in case of liquids) and/or 0.02-0.05 gm (in case of solids) of the test material was used for the study.

6.0 Procedure:

- Subject was requested to perform the re-challenge 4-6 weeks after initial challenge.
- Subject was requested to bathe or wash as usual before arrival at the facility.
- Patches containing the test materials (original test product), if applicable, were then affixed directly to the

skin of the intrascapular regions of the back to an area not previously patched, to the right or left of the midline and subjects were dismissed with instructions not to wet or expose the test area to direct sunlight.

- Sponsor may submit additional samples for testing. These may include but not limited to: different batches, individual ingredients, alternate samples from same batch, etc.
- Subject was instructed to remove patches 24 hours after application for the remainder of the study.
- Test sites were evaluated by trained laboratory personnel 48 and 96 hours after application.
- In the event of an adverse reaction, the area of erythema and edema were measured. Edema is estimated by the evaluation of the skin with respect to the contour of the unaffected normal skin.
- Subject was instructed to report any delayed reactions that might occur after the final reading.
- Clients will be notified immediately in the case of an adverse reaction and a determination is made as to treatment program if necessary.

7.0 Scoring:

Scoring scale and definition of symbols shown below are based on the scoring scheme according to the International Contact Dermatitis Research Group scoring scale [Rietschel, R.L., Fowler, J.F., Ed., Fisher's Contact Dermatitis (fourth ed.). Baltimore, Williams & Wilkins, 1995] listed below:

0 no reaction (negative)
1 erythema throughout at least ¾ of patch area
2 erythema and induration throughout at least ¾ of patch area
3 erythema, induration and vesicles
4 erythema, induration and bullae

D Site discontinued
Dc Subject discontinued voluntarily
Dcl Subject discontinued per Investigator

NOTE: Clinical evaluations are performed by a BCS investigator or designee trained in the clinical evaluation of the skin. Whenever feasible, the same individual will do the scoring of all the subjects throughout the study and will be blinded to the treatment assignments and any previous scores.

8.0 Results:

Subject 3000292:

No.	Accession No.	Test Material Description	Patch Description	Challenge 1	Challenge 2
1	1165900	[redacted] Formula Number: 66IM-16, Lot/Batch# N/A	Occlusive	0	0

9.0 Evaluation Period:

Re-challenge performed on September 6, 2021 to September 10, 2021.

10.0 Observations:

No adverse reactions of any kind were reported during the course of this study.

11.0 Study Archives:

All original samples, raw data sheets, technician's notebooks, correspondence files and copies of final reports and remaining specimens will be maintained on premises of BCS in limited access storage files marked "Archive".

12.0 Conclusions:

Under the conditions of the re-challenge study, there was no indication of a potential to elicit sensitization (contact allergy) noted for [redacted] Formula Number: 66IM-16, Lot/Batch# N/A; Accession No. 1165900.

[redacted]

[redacted]

BioScreen® Testing Services, Inc.
3892 Del Amo Boulevard • Torrance, California 90503
(310) 214-0043 • Fax(310) 370-3642
Web Site: www.bioscreen.com • E-Mail: info@bioscreen.com

50 SUBJECT HUMAN REPEAT INSULT PATCH TEST FOR SKIN IRRITATION AND SKIN SENSITIZATION EVALUATION

Date: July 1, 2015
Revision #1 Date: July 1, 2015

BCS Study No.: 15-404A

Sponsor: [redacted]

1.0 Objective: To determine the irritation and sensitization (contact allergy) potential of a test material after repeated application to the skin of human subjects.

2.0 Test Material:
2.1 Test Material Description:

The use concentration for IPC in this study is 0.0075%.

Date Received: May 12, 2015

Received From: [redacted]

Number Of Test Samples Received: 1

Label On Test Samples: [redacted]***, Lab Ref: 40-56A, Date: 05-04-15***

Accession No.: 900000

2.2 Handling:

Upon arrival at BioScreen Clinical Services (BCS) the test material was assigned a unique laboratory code number and entered into a daily log identifying the lot number, sample description, sponsor, date received and tests requested.

Samples will be retained for a period of thirty (30) days beyond submission of final report unless otherwise specified

by the sponsor or, if sample is known to be in support of governmental applications, in which case representative retained samples are kept two (2) years beyond final report submission.

Sample disposition will be conducted in compliance with appropriate federal, state and local ordinances.

3.0 Panel Selection:

3.1 Standards for Inclusion in a Study:

- Individuals who were not currently under a doctor's care.
- Individuals who were free of any dermatological or systemic disorder that would interfere with the results, at the discretion of the Investigator.
- Individuals who were free of any acute or chronic disease that would interfere with or increase the risk of study participation.
- Individuals who completed a preliminary medical history form mandated by BCS and were in general good health.
- Individuals who read, understood and signed an informed consent document relating to the specific type of study.
- Individuals who were able to cooperate with the Investigator and research staff, and were willing to have test materials applied according to the protocol, and complete the full course of the study.

3.2 Standards for Exclusion from a Study:

- Individuals who were under 18 years of age.
- Individuals who were currently under a doctor's care.
- Individuals who were currently taking any medication (topical or systemic) that might mask or interfere with the test results.
- Individuals who had a history of any acute or chronic disease that might interfere with or increase the risk associated with study participation.
- Individuals who were diagnosed with chronic skin allergies.
- Female volunteers who indicated that they were pregnant or nursing.

3.3 Recruitment:

Panel selection was accomplished by advertisements in local periodicals, community bulletin boards, phone

solicitation, electronic media or any combination thereof.

3.4 Informed Consent and Medical History Forms:

An informed consent was obtained from each volunteer prior to initiating the study describing reasons for the study, possible adverse effects, associated risks and potential benefits of the treatment and their limits of liability. Panelists signed and dated the informed consent document to indicate their authorization to proceed and acknowledge their understanding of the contents. Each subject was assigned a permanent identification number and completed an extensive medical history form. These forms along with the signed consent forms are available for inspection on the premises of BCS only. [Reference 21 CFR Ch. 1 Part 50, Subpart B]

The parties agree to comply with applicable state and federal privacy laws for the use and disclosure of a subject's personal health information by taking reasonable steps to protect the confidentiality of this information. This obligation shall survive the termination or expiration of this Agreement.

4.0 Population Demographics:

Number of subjects enrolled	67
Number of subjects completing study	58
Age Range	18-62
Sex	
Male	19
Female	39
Fitzpatrick Skin Type*	
1 – always burn, does not tan	0
2 – burn easily, tan slightly	21
3 – burn moderately, tan progressively	14
4 – burn a little, always tan	7
5 – rarely burn, tan intensely	12
6 – never burn, tan very intensely	4

*[Agache P., Hubert P.. Measuring the skin. (p. 473, table 48.1) Springer-Verlag Berlin Heidelberg, 2004, (p. 473, table 48.1)]

5.0 Equipment:

Test materials to be tested under occlusive conditions were placed on an 8-millimeter aluminum Finn Chamber® (Epitest Ltd. Oy, Tuusula, Finland) supported on Scanpor® Tape (Norgesplaster A/S, Kristiansand, Norway) or an 8-millimeter filter paper coated aluminum Finn Chamber® AQUA supported on a thin flexible transparent polyurethane rectangular film coated on one side with a medical grade acrylic adhesive, consistent with adhesive used in state-of-the-art hypoallergenic surgical tapes or a 7mm IQ-ULTRA® closed cell system which is made of additive-free polyethylene plastic foam with a filter paper incorporated (It is supplied in units of 10 chambers on a hypoallergenic non woven adhesive tape; the width of the tape is 52mm and the length is 118mm) or other equivalents.

Test materials to be tested under semi-occlusive conditions were placed on a 23-millimeter hypoallergenic skin bandage (Curad Sensitive Skin®, Beiersdorf Inc., Wilton CT) or on a 7.5mm filter paper disc affixed to a strip of hypoallergenic tape (Johnson & Johnson 1 inch First Aid Cloth Tape).

Test materials to be tested in an open patch were applied and rubbed directly onto the back of the subject.

Approximately 0.02-0.05 mL (in case of liquids) and/or 0.02-0.05 gm (in case of solids) of the test material was used for the study. Liquid test material was dispensed on a 7.5mm paper disk, which fit in the Finn Chamber.

6.0 Procedure:

- Subjects were requested to bathe or wash as usual before arrival at the facility.
- Patches containing the test material were then affixed directly to the skin of the intrascapular regions of the back, to the right or left of the midline and subjects were dismissed with instructions not to wet or expose the test area to direct sunlight.
- Patches remained in place for 48 hours after the first application. Subjects were instructed not to remove the patches prior to their 48 hour scheduled visit. Thereafter, subjects were instructed to remove patches 24 hours after application for the remainder of the study.

- This procedure was repeated until a series of nine (9) consecutive, 24-hour exposures had been made three (3) times a week for three (3) consecutive weeks.
- Prior to each reapplication, the test sites evaluated by trained laboratory personnel.
- Following a 10-14 day rest period a retest/challenge dose was applied once to a previously unexposed test site. Test sites were evaluated by trained laboratory personnel 48 and 96 hours after application.
- In the event of an adverse reaction, the area of erythema and edema were measured. Edema is estimated by the evaluation of the skin with respect to the contour of the unaffected normal skin.
- Subjects were instructed to report any delayed reactions that might occur after the final reading.
- Clients will be notified immediately in the case of an adverse reaction and a determination is made as to treatment program if necessary.

7.0 Scoring:

Scoring scale and definition of symbols shown below are based on the scoring scheme according to the International Contact Dermatitis Research Group scoring scale [Rietschel, R.L., Fowler, J.F., Ed., Fisher's Contact Dermatitis (fourth ed.). Baltimore, Williams & Wilkins, 1995] listed below:

0 no reaction (negative)
1 erythema throughout at least ¾ of patch area
2 erythema and induration throughout at least ¾ of patch area
3 erythema, induration and vesicles
4 erythema, induration and bullae

D Site discontinued
Dc Subject discontinued

NOTE: Clinical evaluations are performed by a BCS investigator or designee trained in the clinical evaluation of the skin. Whenever feasible, the same individual will do the scoring of all the subjects throughout the study and will be blinded to the treatment assignments and any previous scores.

8.0 Results:

Accession No.: 900000
Test Material Description: [redacted], ***Lab Ref: 40-56A, Date: 05-04-15***
Patch Description: Occlusive

Subject Information					Induction									Challenge	
No.	ID	Sex	Age	Skin Type	1	2	3	4	5	6	7	8	9	1	2
1	9238	F	28	2	0	0	0	0	0	0	0	0	0	0	0
2	10171	M	49	2	0	0	0	0	0	0	0	0	0	0	0
3	10709	F	53	4	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc
4	10884	F	50	5	0	0	0	0	0	0	0	0	0	0	0
5	10922	F	52	2	0	0	0	0	0	0	0	0	0	0	0
6	10940	M	47	2	0	1	0	0	0	0	0	0	0	0	0
7	10982	F	51	3	0	0	0	0	0	0	0	0	0	0	0
8	11115	F	26	5	0	0	0	0	0	0	0	0	0	0	0
9	11166	M	43	4	0	0	0	0	0	0	0	0	0	0	0
10	11638	M	60	3	0	0	0	0	0	0	0	0	0	0	0
11	11726	F	58	2	0	0	0	0	0	0	0	0	0	0	0
12	11732	F	24	3	0	0	0	0	0	0	0	0	0	0	0
13	12107	M	54	3	0	0	0	0	0	0	0	0	0	0	0
14	12213	F	44	4	0	0	0	0	0	0	0	0	0	0	0
15	12292	M	54	6	0	0	0	0	0	0	0	0	0	0	0
16	12497	F	39	4	0	0	0	0	0	0	0	0	0	0	0
17	14415	F	53	2	0	0	0	0	0	0	0	0	0	0	0
18	14511	M	32	2	0	0	0	0	0	0	0	0	0	0	0
19	14690	F	51	2	0	0	0	0	0	0	0	0	0	0	0
20	14693	F	62	2	0	0	0	0	0	0	0	0	0	0	0
21	14827	M	54	2	0	0	0	0	0	0	0	0	0	0	0
22	14831	F	29	3	0	0	0	0	0	0	0	0	0	0	0
23	14909	F	45	5	0	0	0	0	0	0	0	0	0	0	0
24	15021	F	39	2	0	0	0	0	0	0	0	0	0	0	0
25	15053	F	20	2	0	0	0	0	0	0	0	0	0	0	0
26	15054	F	42	2	0	0	0	0	0	0	0	0	0	0	0
27	15146	F	22	2	0	0	0	0	0	0	0	0	0	0	0
28	15229	M	51	2	0	0	0	0	0	0	0	0	0	0	0
29	15512	M	31	3	0	0	0	0	0	0	0	0	0	0	0
30	15623	F	54	3	0	0	0	0	0	0	0	0	0	0	0
31	15767	F	57	2	0	0	0	0	0	0	0	0	0	0	0
32	15954	F	43	3	0	0	0	0	0	0	0	0	0	0	0
33	15984	F	38	5	0	0	0	0	0	0	0	0	0	0	0

Subject Information					Induction									Challenge	
No.	ID	Sex	Age	Skin Type	1	2	3	4	5	6	7	8	9	1	2
34	16194	F	53	6	0	0	0	0	0	0	0	0	0	0	0
35	16239	M	21	2	0	0	0	0	0	0	0	0	0	0	0
36	16543	F	46	4	0	0	0	0	0	0	0	0	0	0	0
37	16567	F	20	4	0	0	0	0	0	0	0	0	0	0	0
38	16907	F	48	5	0	0	0	0	0	0	0	0	0	0	0
39	16993	F	52	4	0	0	0	0	0	0	0	0	0	0	0
40	17548	M	22	5	0	0	0	0	0	0	0	0	0	0	0
41	17633	F	19	4	0	0	0	0	0	0	0	0	0	0	0
42	17743	M	46	5	0	0	0	0	0	0	0	0	0	0	0
43	17843	F	54	2	0	0	0	0	0	0	0	0	0	0	0
44	17853	F	35	2	0	0	0	0	0	0	0	0	0	0	0
45	17876	F	29	3	0	0	0	0	0	0	0	0	0	0	0
46	17891	M	53	5	0	0	0	0	0	0	0	0	0	0	0
47	18621	F	34	3	0	0	0	0	0	0	0	0	0	0	0
48	18622	F	51	2	0	0	0	0	0	0	0	0	0	0	0
49	18966	M	38	6	0	0	0	0	0	0	0	0	0	0	0
50	19233	M	49	2	0	0	0	0	0	0	0	0	0	0	0
51	19384	M	18	3	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc
52	19525	M	42	5	0	0	0	0	0	0	0	0	0	0	0
53	19734	M	45	2	0	0	0	0	0	0	0	0	0	0	0
54	19763	F	43	3	0	0	0	0	0	0	0	0	0	0	0
55	19777	M	44	2	0	0	0	1	0	0	0	0	0	0	0
56	19778	F	21	4	0	0	0	0	0	0	0	0	0	0	0
57	19787	F	45	5	0	0	0	0	0	0	0	0	0	0	0
58	20003	M	26	2	0	0	0	0	0	0	0	0	0	0	0
59	20156	F	57	6	0	0	0	0	0	0	0	0	0	0	0
60	20311	F	18	3	0	0	0	0	0	0	0	0	0	0	0
61	20388	F	51	3	0	0	0	0	0	0	0	0	0	0	0
62	20606	F	60	2	0	0	0	0	0	0	0	0	0	0	0
63	20654	F	57	3	0	0	0	0	0	0	0	0	0	0	0
64	20655	F	55	5	0	0	0	0	0	0	0	0	0	0	0
65	20696	M	50	5	0	0	0	0	0	0	0	0	0	0	0
66	20698	F	48	5	0	0	0	0	0	0	0	0	0	0	0
67	20863	F	20	3	0	0	0	0	0	0	0	0	0	0	0

9.0 Evaluation Period:

The study was conducted from May 18, 2015 to June 26, 2015.

10.0 Observations:

No adverse reactions of any kind were reported during the course of this study.

11.0 Study Archives:

All original samples, raw data sheets, technician's notebooks, correspondence files and copies of final reports and remaining specimens will be maintained on premises of BCS in limited access storage files marked "Archive".

12.0 Conclusions:

Under conditions of the study, there were no identifiable signs or symptoms of primary irritation or sensitization (contact allergy) noted for [redacted]***, Lab Ref: 40-56A, Date: 05-04-15***; Accession No. 900000.

[redacted]

[redacted]

BioScreen® Testing Services, Inc.
3892 Del Amo Boulevard • Torrance, California 90503
(310) 214-0043
Web Site: www.bioscreen.com • E-Mail: info@bioscreen.com

50 SUBJECT HUMAN REPEAT INSULT PATCH TEST FOR SKIN IRRITATION AND SKIN SENSITIZATION EVALUATION

Date: February 21, 2018

BCS Study No.: 18-501A

Sponsor: [redacted]

1.0 Objective: To determine the irritation and sensitization (contact allergy) potential of a test material after repeated application to the skin of human subjects.

2.0 Test Material:

2.1 Test Material Description:

Date Received: December 26, 2017

Received From: [redacted]

The use concentration for IPC in this study is 0.0075%.

Number Of Test Samples Received: 1

Label On Test Samples: [redacted]
52-33/LL: 52-40

Accession No.: 1040553

2.2 Handling:

Upon arrival at BioScreen Clinical Services (BCS) the test material was assigned a unique laboratory code number and entered into a daily log identifying the lot number, sample description, sponsor, date received and tests requested.

Samples will be retained for a period of thirty (30) days beyond submission of final report unless otherwise specified

by the sponsor or, if sample is known to be in support of governmental applications, in which case representative retained samples are kept two (2) years beyond final report submission.

Sample disposition will be conducted in compliance with appropriate federal, state and local ordinances.

3.0 Panel Selection:

3.1 Standards for Inclusion in a Study:

- Individuals who were not currently under a doctor's care.
- Individuals who were free of any dermatological or systemic disorder that would interfere with the results, at the discretion of the Investigator.
- Individuals who were free of any acute or chronic disease that would interfere with or increase the risk of study participation.
- Individuals who completed a preliminary medical history form mandated by BCS and were in general good health.
- Individuals who read, understood and signed an informed consent document relating to the specific type of study.
- Individuals who were able to cooperate with the Investigator and research staff, and were willing to have test materials applied according to the protocol, and complete the full course of the study.

3.2 Standards for Exclusion from a Study:

- Individuals who were under 18 years of age.
- Individuals who were currently under a doctor's care.
- Individuals who were currently taking any medication (topical or systemic) that might mask or interfere with the test results.
- Individuals who had a history of any acute or chronic disease that might interfere with or increase the risk associated with study participation.
- Individuals who were diagnosed with chronic skin allergies.
- Female volunteers who indicated that they were pregnant or nursing.

3.3 Recruitment:

Panel selection was accomplished by advertisements in local periodicals, community bulletin boards, phone solicitation, electronic media or any combination thereof.

3.4 Informed Consent and Medical History Forms:

An informed consent was obtained from each volunteer prior to initiating the study describing reasons for the study, possible adverse effects, associated risks and potential benefits of the treatment and their limits of liability. Panelists signed and dated the informed consent document to indicate their authorization to proceed and acknowledge their understanding of the contents. Each subject was assigned a permanent identification number and completed an extensive medical history form. These forms along with the signed consent forms are available for inspection on the premises of BCS only. [Reference 21 CFR Ch. 1 Part 50, Subpart B]

The parties agree to comply with applicable state and federal privacy laws for the use and disclosure of a subject's personal health information by taking reasonable steps to protect the confidentiality of this information. This obligation shall survive the termination or expiration of this Agreement.

4.0 Population Demographics:

Number of subjects enrolled	55
Number of subjects completing study	54
Age Range	18-64
Sex	
Male	5
Female	49
Fitzpatrick Skin Type*	
1 – always burn, does not tan	0
2 – burn easily, tan slightly	0
3 – burn moderately, tan progressively	54
4 – burn a little, always tan	0
5 – rarely burn, tan intensely	0
6 – never burn, tan very intensely	0

*[Agache P., Hubert P.. Measuring the skin. (p. 473, table 48.1) Springer-Verlag Berlin Heidelberg, 2004, (p. 473, table 48.1)]

5.0 Equipment:

Test materials to be tested under occlusive conditions were placed on an 8-millimeter aluminum Finn Chamber® (Epitest Ltd. Oy, Tuusula, Finland) supported on Scanpor® Tape (Norgesplaster A/S, Kristiansand, Norway) or an 8-millimeter filter paper coated aluminum Finn Chamber® AQUA supported on a thin flexible transparent polyurethane rectangular film coated on one side with a medical grade acrylic adhesive, consistent with adhesive used in state-of-the-art hypoallergenic surgical tapes or a 7mm IQ-ULTRA® closed cell system which is made of additive-free polyethylene plastic foam with a filter paper incorporated (It is supplied in units of 10 chambers on a hypoallergenic non woven adhesive tape; the width of the tape is 52mm and the length is 118mm) or other equivalents.

Test materials to be tested under semi-occlusive conditions were placed on a test strip with a Rayon/Polypropylene pad or on a 7.5mm filter paper disc affixed to a strip of hypoallergenic tape (Johnson & Johnson 1 inch First Aid Cloth Tape).

Test materials to be tested in an open patch were applied and rubbed directly onto the back of the subject.

Approximately 0.02-0.05 mL (in case of liquids) and/or 0.02-0.05 gm (in case of solids) of the test material was used for the study. Liquid test material was dispensed on a 7.5mm paper disk, which fit in the Finn Chamber.

6.0 Procedure:

- Subjects were requested to bathe or wash as usual before arrival at the facility.
- Patches containing the test material were then affixed directly to the skin of the intrascapular regions of the back, to the right or left of the midline and subjects were dismissed with instructions not to wet or expose the test area to direct sunlight.
- Patches remained in place for 48 hours after the first application. Subjects were instructed not to remove the patches prior to their 48 hour scheduled visit. Thereafter,

subjects were instructed to remove patches 24 hours after application for the remainder of the study.
- This procedure was repeated until a series of nine (9) consecutive, 24-hour exposures had been made three (3) times a week for three (3) consecutive weeks.
- Prior to each reapplication, the test sites evaluated by trained laboratory personnel.
- Following a 10-14 day rest period a retest/challenge dose was applied once to a previously unexposed test site. Test sites were evaluated by trained laboratory personnel 48 and 96 hours after application.
- In the event of an adverse reaction, the area of erythema and edema were measured. Edema is estimated by the evaluation of the skin with respect to the contour of the unaffected normal skin.
- Subjects were instructed to report any delayed reactions that might occur after the final reading.
- Clients will be notified immediately in the case of an adverse reaction and a determination is made as to treatment program if necessary.

7.0 Scoring:

Scoring scale and definition of symbols shown below are based on the scoring scheme according to the International Contact Dermatitis Research Group scoring scale [Rietschel, R.L., Fowler, J.F., Ed., Fisher's Contact Dermatitis (fourth ed.). Baltimore, Williams & Wilkins, 1995] listed below:

0 no reaction (negative)
1 erythema throughout at least ¾ of patch area
2 erythema and induration throughout at least ¾ of patch area
3 erythema, induration and vesicles
4 erythema, induration and bullae

D Site discontinued
Dc Subject discontinued voluntarily
DcI Subject discontinued per Investigator

NOTE: Clinical evaluations are performed by a BCS investigator or designee trained in the clinical evaluation of the skin. Whenever feasible, the same individual will do the scoring of all the subjects throughout the study and will be blinded to the treatment assignments and any previous scores.

8.0 Results:

Accession No.: 1040553
Test Material Description: [redacted]: 52-33/LL: 52-40
Patch Description: Occlusive

Subject Information					Induction									Challenge	
No.	ID	Sex	Age	Skin Type	1	2	3	4	5	6	7	8	9	1	2
1	3000073	F	38	3	0	0	0	0	0	0	0	0	0	0	0
2	3000078	F	51	3	0	0	0	0	0	0	0	0	0	0	0
3	3000142	F	37	3	0	0	0	0	0	0	0	0	0	0	0
4	3000162	F	62	3	0	0	0	0	0	0	0	0	0	0	0
5	3000257	F	51	3	0	0	0	0	0	0	0	0	0	0	0
6	3000273	F	53	3	0	0	0	0	0	0	0	0	0	0	0
7	3000336	F	33	3	0	0	0	0	0	0	0	0	0	0	0
8	3000565	F	57	3	0	0	0	0	0	0	0	0	0	0	0
9	3000573	F	39	3	0	0	0	0	0	0	0	0	0	0	0
10	3000580	F	47	3	0	0	0	0	0	0	0	0	0	0	0
11	3000709	F	38	3	0	0	0	0	0	0	0	0	0	0	0
12	3000835	F	53	3	0	0	0	0	0	0	0	0	0	0	0
13	3000909	F	40	3	0	0	0	0	0	0	0	0	0	0	0
14	3000916	F	44	3	0	0	0	0	0	0	0	0	0	0	0
15	3000940	F	20	3	0	0	0	0	0	0	0	0	0	0	0
16	3001968	F	20	3	0	0	0	0	0	0	0	0	0	0	0
17	3002051	F	27	3	0	0	0	0	0	0	0	0	0	0	0
18	3003133	F	57	3	0	0	0	0	0	0	0	0	0	0	0
19	3003214	F	52	3	0	0	0	0	0	0	0	0	0	0	0
20	3003222	F	45	3	0	0	0	0	0	0	0	0	0	0	0
21	3005190	F	52	3	0	0	0	0	0	0	0	0	0	0	0
22	3005197	M	64	3	0	0	0	0	0	0	0	0	0	0	0
23	3005199	F	46	3	0	0	0	0	0	0	0	0	0	0	0
24	3005204	F	38	3	0	0	0	0	0	0	0	0	0	0	0
25	3005209	F	30	3	0	0	0	0	0	0	0	0	0	0	0
26	3005211	F	53	3	0	0	0	0	0	0	0	0	0	0	0
27	3006284	F	26	3	0	0	0	0	0	0	0	0	0	0	0
28	3007289	F	56	3	0	0	0	0	0	0	0	0	0	0	0
29	3007297	F	40	3	0	0	0	0	0	0	0	0	0	0	0
30	3007310	F	38	3	0	0	0	0	0	0	0	0	0	0	0
31	3007311	F	18	3	0	0	0	0	0	0	0	0	0	0	0
32	3009328	F	18	3	0	0	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc
33	3009329	F	28	3	0	0	0	0	0	0	0	0	0	0	0
34	3009334	F	44	3	0	0	0	0	0	0	0	0	0	0	0

Subject Information					Induction									Challenge	
No.	ID	Sex	Age	Skin Type	1	2	3	4	5	6	7	8	9	1	2
35	3009337	M	35	3	0	0	0	0	0	0	0	0	0	0	0
36	3009342	M	31	3	0	0	0	0	0	0	0	0	0	0	0
37	3009427	F	25	3	0	0	0	0	0	0	0	0	0	0	0
38	3009466	F	26	3	0	0	0	0	0	0	0	0	0	0	0
39	3009479	F	59	3	0	0	0	0	0	0	0	0	0	0	0
40	3009484	F	35	3	0	0	0	0	0	0	0	0	0	0	0
41	3009486	F	32	3	0	0	0	0	0	0	0	0	0	0	0
42	3009489	F	57	3	0	0	0	0	0	0	0	0	0	0	0
43	3009501	F	23	3	0	0	0	0	0	0	0	0	0	0	0
44	3009502	F	31	3	0	0	0	0	0	0	0	0	0	0	0
45	3009503	F	30	3	0	0	0	0	0	0	0	0	0	0	0
46	3009504	F	37	3	0	0	0	0	0	0	0	0	0	0	0
47	3009510	F	31	3	0	0	0	0	0	0	0	0	0	0	0
48	3009511	F	57	3	0	0	0	0	0	0	0	0	0	0	0
49	3009530	F	19	3	0	0	0	0	0	0	0	0	0	0	0
50	3009549	M	20	3	0	0	0	0	0	0	0	0	0	0	0
51	3009552	F	35	3	0	0	0	0	0	0	0	0	0	0	0
52	3009556	F	34	3	0	0	0	0	0	0	0	0	0	0	0
53	3010683	F	23	3	0	0	0	0	0	0	0	0	0	0	0
54	3010684	M	23	3	0	0	0	0	0	0	0	0	0	0	0
55	3010685	F	33	3	0	0	0	0	0	0	0	0	0	0	0

9.0 Evaluation Period:

The study was conducted from January 8, 2018 to February 16, 2018.

10.0 Observations:

No adverse reactions of any kind were reported during the course of this study.

There were five (5) subjects with a Grade 1 reaction and one (1) subject with a delayed Grade 1 reaction to the positive control (2.0% Sodium Lauryl Sulfate Solution).

No subjects showed any signs of reaction to the negative control (DI Water).

11.0 Study Archives:

All original samples, raw data sheets, technician's notebooks, correspondence files and copies of final reports and remaining specimens will be maintained on premises of BCS in limited access storage files marked "Archive".

12.0 Conclusions:

Under the conditions of the study, there was no indication of a potential to elicit dermal irritation or sensitization (contact allergy) noted for [redacted] 52-33/LL: 52-40; Accession No. 1040553.

[redacted]

[redacted]

Stephens
Excellence in Research

A Single-Center Clinical Study to Evaluate the Safety and Efficacy of [redacted] When Used on Eyelashes and Eyebrows

Prepared for:

[redacted]

Thomas J. Stephens & Associates, Inc.

Stephens Study Number: C18-D094

[redacted] **Study Number: 2018TSA025**

[redacted]

1801 North Glenville Drive Suite 200 Richardson, Texas 75081-1906
T 972.392.1529 F 972.392.2347 W www.stephens-associates.com

[redacted]
Thomas J. Stephens & Associates, Inc.
Stephens Study Number: C18-D094
[redacted] Study Number: 2018TSA025
Final Report 25 Apr 2019

TABLE OF CONTENTS

LIST OF TABLES

PURPOSE

Visual acuity tests and slit-lamp ophthalmological examinations are well-established methods for determining the ocular and eye area safety of topical products after a period of daily use.

This single-center clinical trial was conducted for [redacted] to assess the safety and efficacy of the Sponsor's eyelash conditioning serum on eyelashes when used over the course of 8 weeks by women in general good health, including those with self-perceived sensitive eyes, contact lens wearers, and regular users of mascara. A secondary objective was to evaluate the use of the serum on eyebrows.

GENERAL INFORMATION

Stephens Study Number:	C18-D094
[redacted] Study Number:	2018TSA025
Test:	A Single-Center Clinical Study to Evaluate the Safety and Efficacy of [redacted] When Used on Eyelashes and Eyebrows
Test Material:	[redacted] (Formula #52-33 / Lot #L3HA8142152)
Investigator: Sub-Investigator/Study Ophthalmologist: Sub-Investigator: Quality Assurance Manager:	[redacted]
Testing and Administrative Facility:	Thomas J. Stephens & Associates, Inc. Texas Research Center 1801 North Glenville Drive, Suite 200 Richardson, TX 75081
Sponsor: Sponsor Representatives:	[redacted]
Testing Start Date:	09 Jul 2018
Testing End Date:	09 Oct 2018

The use concentration for IPC in this study is 0.0075%.

SUMMARY

This single-center clinical trial was conducted for [redacted] to assess the safety and efficacy of the Sponsor's eyelash conditioning serum on eyelashes when used over the course of 8 weeks by women in general good health, including those with self-perceived sensitive eyes, contact lens wearers, and regular users of mascara. A secondary objective was to evaluate the use of the serum on eyebrows.

Out of 56 subjects who completed study participation, 53 subjects (94.6%) wear mascara at least 4 days per week, 50 subjects (89.3%) have self-perceived sparse eyebrows, 19 subjects (33.9%) wear contact lenses regularly, and 18 subjects (32.1%) have self-perceived sensitive eyes.

During the course of the study, subjects applied the test material [redacted] (Formula #52-33 / Lot #L3HA8142152)] to the upper eyelashes and eyebrows once per day at night, as directed.

Clinical evaluations were conducted at visit 1 (baseline), visit 2 (week 1), visit 3 (week 2), visit 4 (week 3), visit 5 (week 4), visit 6 (week 5), visit 7 (week 6), visit 8 (week 7), and visit 9 (week 8). Subjects participated in the following procedures at each time point (unless otherwise indicated):

- Visual Acuity Test
  At baseline and weeks 4 and 8, visual acuity was assessed separately for each subject's right and left eyes. During the test, subjects wore their normal corrective eyewear (glasses or contact lenses), if any.

- Slit-Lamp Ophthalmological Examination
  The Study Ophthalmologist performed a slit-lamp examination separately for each subject's right and left eyes. The exam included grading for bulbar conjunctival irritation (hyperemia, edema, erosions, and follicles), tarsal conjunctival irritation (hyperemia, edema, and follicles), lacrimation, contact lens deposits (if applicable), and fluorescein staining (the level of brightness and the percent of the eye that the stain covers).

  Additionally, subjects assessed their right and left eyes separately for burning/stinging, itching, foreign body sensation, and soreness.

- Cutaneous Tolerance Evaluation
  Local cutaneous tolerability was evaluated by assessing the signs and symptoms of objective and subjective irritation separately on the left and right eyes (around the eyelash lines on upper and lower lids) and in and around the eyebrows (left and right eyebrows evaluated together). The following irritation parameters were evaluated:
  - Objective irritation (clinically graded): erythema, dryness, and edema
  - Subjective irritation (assessed by subjects): burning, stinging, itching, tightness, and tingling

- VISIA CR Imaging Procedures
  At baseline and weeks 2, 4, 6, and 8, a total of 3 full-face digital images were taken of each subject's face (left, center, and right views) using the VISIA CR photo station (Canfield Imaging Systems, Fairfield, New Jersey) with a Canon Mark II digital SLR camera (Canon Incorporated, Tokyo, Japan) under the following lighting conditions: standard 1 (visible [bright]), standard 2 (visible), and cross-polarized.

[redacted]

SUMMARY (continued)

- Photo Comparison Grading of Images
  At weeks 2, 4, 6, and 8, a clinical grader compared VISIA CR images from each visit to images from baseline. The grader assessed each subject's lashes and brows separately for the following parameters: longer-looking, darker-looking, and fuller-looking. The left and right sides were graded separately.

Overall Conclusions

Overall results from this single-center clinical trial indicate that the Sponsor's eyelash conditioning serum [redacted] (Formula #52-33 / Lot #L3HA8142152)] did not cause any statistically significant worsening in visual acuity test or slit-lamp ophthalmological examination scores including subjective sensations [except fluorescein staining at week 1 on the right eye] when used over the course of 8 weeks by women in general good health, including those with self-perceived sensitive eyes, contact lens wearers, and regular users of mascara. Additionally, there was no statistically significant worsening in cutaneous tolerance evaluation scores for the eyes or eyebrows. Photo comparisons also identified significant improvement in lashes and eyebrows looking longer/darker/fuller.

A total of 4 subjects experienced AEs determined to be related to the test material; 2 subjects experienced mild eye pain, dry eye, and/or ocular hyperaemia **possibly** related to the test material; 1 subject with self-perceived sensitive eyes experienced moderate eye pain and eye irritation **probably** related to the test material; and 1 subject experienced mild ocular hyperaemia and erythema of the eyelids, and moderate eye irritation **definitely** related to the test material.

Results of the visual acuity test showed no statistically significant changes from baseline in scores at weeks 4 or 8 for the left eye, right eye, or average of both eyes.

Results of the slit-lamp ophthalmological examination showed no statistically significant changes from baseline in scores for bulbar conjunctival irritation (hyperemia, edema, erosions, and follicles), tarsal conjunctival irritation (hyperemia, edema, and follicles), lacrimation, contact lens deposits (if applicable), or any subjective sensations (burning/stinging, itching, foreign body sensation, and soreness) at any post-baseline time point (weeks 1, 2, 3, 4, 5, 6, 7, and 8) for the left eye, right eye, or average of both eyes. There was a statistically significant worsening in fluorescein staining (the level of brightness and the percent of the eye that the stain covers) at week 1 for the right eye when compared with baseline. The worsening was transient, as there were no statistically significant changes from baseline at weeks 2 through 8 for the level of brightness and the percent of the eye that the stain covers, and not clinically relevant, as the increase was very small and only in the right eye.

Results of the cutaneous tolerance evaluation showed no statistically significant changes from baseline for erythema, dryness, edema, burning, stinging, itching, tightness, or tingling at any post-baseline time point for the left eye, right eye, or eyebrows.

[redacted]

[redacted]

STORAGE, HANDLING, AND DOCUMENTATION OF TEST MATERIALS

The receipt of study materials by Thomas J. Stephens & Associates, Inc. was documented in a study material log, which serves as a permanent record of the receipt, storage, return, and disposition of all study materials. All study materials are kept in a locked product-storage room accessible to designated staff members only. At the Sponsor's request, used test materials will be destroyed and unused test materials will be returned to the Sponsor according to Stephens' Standard Operating Procedures (SOPs).

TEST MATERIAL DESCRIPTIONS

Table 1 presents the test material description. Each test material was labeled with the assigned test material identification number (TMIN) and product identification (ID).

Table 1: Test Material Description

TMIN	Product ID	Physical Properties	Frequency
0292-18C	[redacted] (Formula #52-33 / Lot #L3HA8142152)	Clear, transparent liquid	Once per day at night

INFORMED CONSENT

Written informed consent conforming to Title 21 Code of Federal Regulations (CFR) 50.25 was obtained from each subject. As part of the informed consent process, the prospective subject was given as much time as needed to read the informed consent form (ICF) and had the opportunity to have any study-related questions answered to their satisfaction prior to signing the ICF. The original signed ICF for each subject participating in the study was retained in the study file and each subject received a copy of the signed ICF. Refer to Appendix VI Sample Forms for a copy of the ICF.

RECORD OF SPONSOR MONITORING VISITS

The Sponsor was permitted to perform site visits during the course of the study and inspect all case report forms (CRFs) and other documentation directly associated with the study. The Sponsor did not perform any study-related site visits.

SUBJECT DISPOSITION AND DEMOGRAPHICS

A summary of subject disposition information is included in Table 2. The demographic information for the per-protocol (PP) population is presented in Table 3. For applicable parameters, the number of subjects in each category is listed with the percentage of total subjects in parentheses. Refer to Appendix VII Screening/Enrollment Log.

Table 2: Subject Disposition

	All Subjects
Enrolled subjects	66
PP population (completed subjects)	56
Discontinued subjects	10
Did not meet protocol criteria[a]	1
Lost to follow-up	4
Subject requested withdrawal	5

[a] Refer to Protocol Deviations for information regarding this discontinued subject.

SUBJECT DISPOSITION AND DEMOGRAPHICS (continued)

Table 3: Summary of Demographic Information – PP Population

	All Subjects	
N	56	
Age (years)		
Mean	53.1	
Standard deviation	8.6	
Minimum	32	
Median	55.5	
Maximum	65	
	N	**(%)**
Sex		
Female	56	(100.0)
Ethnicity		
Hispanic or Latino	2	(3.6)
Not Hispanic or Latino	54	(96.4)
Race		
Asian	2	(3.6)
Black or African American	22	(39.3)
White or Caucasian	31	(55.4)
Multiracial	1	(1.8)
Fitzpatrick skin type		
I	3	(5.4)
II	17	(30.4)
III	13	(23.2)
IV	1	(1.8)
V	20	(35.7)
VI	2	(3.6)
Wear mascara at least 4 days per week	53	(94.6)
Self-perceived sparse eyebrows	50	(89.3)
Regular wearers of contact lens	19	(33.9)
Self-perceived sensitive eyes	18	(32.1)

ADVERSE EVENTS

An adverse event (AE) is defined as any untoward medical occurrence in a clinical investigation where a subject is administered any product or medical device, regardless of causal relationship with the test article. In general, the most common side effects associated with topical products are mild irritation, which may include (but are not limited to) subjective sensations (such as itching, burning, stinging, tingling), scaling/dryness, and redness. Additionally, products applied to the eye areas have the potential to cause eye watering/tearing, redness of the eyes, corneal erosions, burning, stinging, and/or a foreign body sensation in the eyes.

Symptoms of irritation, including the examples above, may not have been treated as adverse reactions if they were mild in nature, even if the symptoms did not resolve over time. Symptoms that were persistent and moderate to severe in nature, or that involved elevation (eg, edema, papules, vesicles, spreading) were considered AEs. The Investigator or designee had the final authorization to determine if a reaction was considered an AE.

A brief summary of the nonserious Aes and serious AE (SAE) recorded during the study are presented in Table 4 and Table 5 on the next page. Refer to Appendix VIII Adverse Event Forms.

ADVERSE EVENTS (continued)

Table 4: Nonserious Adverse Events

Subject	Adverse Event (Location)	Date Started	Date Ended	Severity	Relationship	Outcome
004	Eye contusion (right eye)	25 Aug 2018	02 Sep 2018	Mild	Unlikely	Resolved
012 (S)	Pain (head, chest, and right arm)	29 Jul 2018	28 Aug 2018	Mild	Unlikely	Resolved
015 (S)	Oedema (lips)	25 Jul 2018	02 Aug 2018	Mild	Unlikely	Resolved
021 (S)	Hordeolum (left upper eyelid)	15 Aug 2018	17 Aug 2018	Mild	Unlikely	Resolved
027	Eye pain (left eye)	06 Aug 2018	11 Aug 2018	Mild	Possible	Resolved
	Ocular hyperaemia (left eye)	06 Aug 2018	18 Aug 2018	Mild	Possible	Resolved
	Dry eye (left eye)	07 Aug 2018	18 Aug 2018	Mild	Possible	Resolved
047	Facial lesion excision (upper lip)	20 Sep 2018	20 Sep 2018	Mild	Unlikely	Resolved
048	Hordeolum (left eye)	06 Sep 2018	09 Sep 2018	Mild	Unlikely	Resolved
053 (S)	Eye pain (right eye)	29 Aug 2018	29 Aug 2018	Moderate	Probable	Resolved
	Eye irritation (right eye)	29 Aug 2018	29 Aug 2018	Moderate	Probable	Resolved
054	Fall (left wrist and left arm)	30 Sep 2018	14 Oct 2018	Mild	Unlikely	Resolved
	Arthralgia (left wrist and left arm)	30 Sep 2018	14 Oct 2018	Mild	Unlikely	Resolved
	Contusion (left wrist and left arm)	30 Sep 2018	07 Oct 2018	Mild	Unlikely	Resolved
060	Laceration (lower lip)	23 Sep 2018	08 Oct 2018	Mild	Unlikely	Resolved
065	Ocular hyperaemia (eyes)	14 Aug 2018	15 Aug 2018	Mild	Possible	Resolved
066	Eye irritation (eyes)	30 Aug 2018	05 Sep 2018	Moderate	Definite	Resolved
	Ocular hyperaemia (eyes)	05 Sep 2018	14 Sep 2018	Mild	Definite	Resolved
	Erythema of eyelid (eyes)	05 Sep 2018	14 Sep 2018	Mild	Definite	Resolved
	Oropharyngeal pain (throat and nose)	30 Sep 2018	07 Oct 2018	Mild	Unlikely	Resolved
	Rhinnorrhoea (throat and nose)	30 Sep 2018	07 Oct 2018	Mild	Unlikely	Resolved

S = Subject with self-perceived sensitive eyes.

Table 5: Serious Adverse Event

Subject	Adverse Event	Date Started	Date Ended	Severity	Relationship	Outcome
033	Pneumonia	13 Sep 2018	25 Sep 2018	Severe	Unlikely	Resolved

PROTOCOL AMENDMENTS

Any changes or formal clarification to the procedures outlined in the protocol were documented as protocol amendments. Notes to file (for internal purposes) were used to identify study discrepancies, provide clarification, or record slight variations for items that did not require a protocol amendment.

There were no amendments to the protocol.

PROTOCOL DEVIATIONS

Any violations to the protocol that may have significantly affected the completeness, accuracy, and/or reliability of the study data or may have affected subjects' rights, safety, or well-being were documented as deviations. Notes to file (for internal clarification purposes) were used to record items that did not qualify as deviations.

The following protocol deviations were recorded over the course of the study:

- Subject 043 was enrolled in the study despite meeting exclusion criterion #15 (microblading on the eyebrows). Subject was discontinued from the study at week 2.
- At baseline, subjects 019, 023, and 037 had VISIA images taken while wearing makeup on their eyebrows. These subjects were excluded from photo comparison grading on the eyebrows.
- The following subjects attended each specified visit outside of the allowed window (actual number of days outside of ±2 days for week 2 or ±3 days for weeks 3-8 is included in parentheses): 033 and 035 (+1) and 044 (+2) at week 2; 055, 063, 066 (+1) at week 4; 030 (+2) at week 6; 033 (+2) at week 8.
- The following subjects missed each specified visit resulting in missing data: 030, 033, and 035 at week 1; 066 at week 5; and 058 at week 7. Subjects verbally confirmed to have continued using the test material nightly. Per the Investigator, subjects were allowed to complete study participation.
- The following subjects did not complete slit-lamp ophthalmological examination resulting in missing data: 033 at week 7 and 060 at week 8.
- The following subjects did not wear a contact lens in the right eye resulting in missing data for contact lens deposit on the right eye at the specified visit: 012 at week 1, and 048 and 053 at week 3.
- At week 4, subjects 008 and 012 participated in slit-lamp ophthalmological examination prior to the visual acuity test.
- At week 1, subject 005 acclimated for only 15 minutes, instead of 20 minutes, after removing makeup upon arrival at the clinic.

The detailed protocol deviation log was reviewed and signed by the Investigator and forwarded to the Sponsor. Refer to Appendix V Protocol Deviation Log.

PROCEDURES AND METHODS

Prior to the start of the study, prospective subjects were screened over the telephone for eligibility criteria. Women between the ages of 18 and 65 years, including those who wear mascara at least 4 days a week, regularly wear contact lenses, have self-perceived sparse eyebrows, or have self-perceived sensitive eyes, were scheduled for eligibility screening at the clinic. Prospective subjects were instructed to wash their face and remove all makeup at least 30 minutes prior to each scheduled visit. Prospective subjects were also instructed to wear the same corrective eyewear (contact lenses or glasses, if applicable) to each scheduled visit.

At visit 1 (baseline), prospective subjects completed the informed consent process and signed the ICF. Prospective subjects who signed this initial paperwork were assigned a screening number and acclimated to ambient temperature and humidity conditions for at least 15 minutes prior to participating in evaluation procedures.

PROCEDURES AND METHODS (continued)

Prospective subjects completed an eligibility and health questionnaire and were evaluated for eligibility criteria, including the following:

- Fitzpatrick Type Skin Classification: Types I-VI qualified
  The Fitzpatrick Skin Classification is based on the skin's unprotected response to the first 30-45 minutes of sun exposure after a winter season without sun exposure. The categories of skin types are as follows:

Type	Physical Characteristics	Skin Reaction to UV
I	White; very fair; red or blonde hair; blue eyes; freckles	Always burns easily; never tans
II	White; fair; red or blonde hair; blue, hazel, or green eyes	Always burns easily; tans minimally
III	Cream white; fair with any eye or hair color; very common	Burns moderately; tans gradually
IV	Brown; typical Mediterranean white skin	Burns minimally; always tans well
V	Dark brown; mid-eastern skin types, black hair, olive skin	Rarely burns; tans profusely
VI	Black; black hair, black eyes, black skin	Never burns; deeply pigmented

  Every effort was made to enroll enough subjects to complete the study with the following approximate percentages for each specified Fitzpatrick skin type: 10% with type I, 80% with types II-V, and 10% with type VI.

- Eyelash Conditions
  Clinically determined minimum to moderate (score of 1-2 where 1=minimal and 4=very marked, according to Global Eyelash Assessment [GEA] scale[1]) score for overall eyelash prominence.

Candidate subjects who passed the eligibility screening participated in the following procedures:

- **Visual Acuity Test**
  Visual acuity was assessed separately for each subject's right and left eyes. During the tests, subjects wore their normal corrective eyewear (glasses or contact lenses), if any. The type of eyewear was recorded using the following associated numbers: 0 = None, 1 = Contacts, and 2 = Glasses.

  Subjects stood away from a manually projected visual acuity chart (Reichert Technologies, LongLife™ Project-O-Chart®, Depew, New York). The manual box projector was formatted to be the equivalent of viewing the chart from 20 feet away. An occluder was placed over 1 eye and the subject read the first fully clear line of the visual acuity chart, followed by each successive line until the subject could not identify 1 or more letters/numbers on the chart. The smallest line that the subject read correctly was considered the visual acuity score and the denominator was recorded. The procedure was repeated for the other eye.

- **Slit-Lamp Ophthalmological Examination**
  The Study Ophthalmologist performed a slit-lamp examination separately for each subject's right and left eyes. The exam included grading for bulbar conjunctival irritation (hyperemia, edema, erosions, and follicles), tarsal conjunctival irritation (hyperemia, edema, and follicles), lacrimation, contact lens deposits (if applicable), and fluorescein staining (the level of brightness and the percent of the eye that the stain covers).

  Subjects who routinely wear contact lenses wore their lenses for the initial eye exam. The lenses were inspected and contact lens deposits were scored by the Study Ophthalmologist. Subjects then removed their lenses prior to having the fluorescein stain placed into the eye. Subjects brought a case, any fluids that were required, and backup eye glasses, as needed for lens removal. Subjects waited at least 15 minutes prior to re-inserting their contact lenses after fluorescein staining, unless the Study Ophthalmologist instructed them otherwise.

PROCEDURES AND METHODS (continued)

- **Slit-Lamp Ophthalmological Examination (continued)**

The following parameters were evaluated using the indicated grading scales (with half-point scores used as necessary to better describe the clinical condition):

Conjunctiva Irritation:
Bulbar hyperemia, edema, erosions, and follicles;
Tarsal hyperemia, edema, and follicles
0 = None
1 = Mild
2 = Moderate
3 = Severe

Lacrimation (Tearing)
0 = None
1 = Mild, occasional tears
2 = Moderate, tears flooding eye but confined to orbit
3 = Severe, tears flooding eye and leaving orbit

Contact Lens Deposits (if applicable)
0 = None
1 = Mild
2 = Moderate
3 = Severe

Fluorescein Staining: Brightness
0 = None
1 = Mild, barely detectable
2 = Moderate, obvious staining
3 = Very strong stain

Fluorescein Staining: Percent of Eye Covered
0 = None, 0%
1 = Mild, up to 30%
2 = Moderate, 30 – 60%
3 = Severe, 60 – 100%

Subjects assessed the following subjective sensations of the eyes using the indicated grading scale (with half-point scores used as necessary to better describe the clinical condition):

Burning/stinging, itching, foreign body sensation, and soreness
0 = None
1 = Mild
2 = Moderate
3 = Severe

Those with a score of 0-1 for all parameters qualified for study participation.

Each candidate subject's eligibility was reviewed, and qualified subjects were enrolled in the study and assigned a subject number.

PROCEDURES AND METHODS (continued)
Subjects participated in the following evaluation procedures:

- **Cutaneous Tolerance Evaluation**
  Local cutaneous tolerability was evaluated by assessing the signs and symptoms of objective and subjective irritation separately on the left and right eyes (around the eyelash lines on upper and lower lids) and in and around the eyebrows (left and right eyebrows evaluated together). These locations were defined as the treatment area. The following irritation parameters were evaluated:
    - Objective irritation (clinically graded): erythema, dryness, and edema
    - Subjective irritation (assessed by subjects): burning, stinging, itching, tightness, and tingling

  For subjective irritation assessments at baseline, subjects reported the degree of any parameters that they typically experience when using a product similar to the test material. At subsequent evaluation time points, subjects reported the degree of symptoms they experienced since the previous evaluation time point.

  Results of the irritation evaluations were recorded using the following scales (with half-point scores assigned as necessary to better describe the clinical condition):

Erythema	
0 = None	No erythema of the treatment area
1 = Mild	Slight, but definite redness of the treatment area
2 = Moderate	Definite redness of the treatment area
3 = Severe	Marked redness of the treatment area

Dryness	
0 = None	No dryness of the treatment area
1 = Mild	Slight, but definite dryness of the treatment area
2 = Moderate	Definite dryness of the treatment area
3 = Severe	Marked dryness of the treatment area

Edema	
0 = None	No edema/swelling of the treatment area
1 = Mild	Slight, but definite edema of the treatment area
2 = Moderate	Definite edema of the treatment area
3 = Severe	Marked edema of the treatment area

Burning	
0 = None	No burning of the treatment area
1 = Mild	Slight burning sensation of the treatment area; not really bothersome
2 = Moderate	Definite warm, burning of the treatment area that is somewhat bothersome
3 = Severe	Hot burning sensation of the treatment area that causes definite discomfort and may interrupt daily activities and/or sleep

Stinging	
0 = None	No stinging of the treatment area
1 = Mild	Slight stinging sensation of the treatment area; not really bothersome
2 = Moderate	Definite stinging of the treatment area that is somewhat bothersome
3 = Severe	Marked stinging sensation of the treatment area that causes definite discomfort and may interrupt daily activities and/or sleep

PROCEDURES AND METHODS (continued)

- **Cutaneous Tolerance Evaluation (continued)**

Itching

0 = None	No itching of the treatment area
1 = Mild	Slight itching sensation of the treatment area; not really bothersome
2 = Moderate	Definite itching of the treatment area that is somewhat bothersome
3 = Severe	Marked itching sensation of the treatment area that causes definite discomfort and may interrupt daily activities and/or sleep

Tightness

0 = None	No skin tightness sensation of the treatment area
1 = Mild	Slight, but definite tightness sensation of the treatment area
2 = Moderate	Definite tightness sensation of the treatment area that is somewhat bothersome
3 = Severe	Marked tightness sensation of the treatment area that causes definite discomfort

Tingling

0 = None	No skin tingling sensation of the treatment area
1 = Mild	Slight, but definite tingling sensation of the treatment area
2 = Moderate	Definite tingling sensation of the treatment area that is somewhat bothersome
3 = Severe	Marked tingling sensation of the treatment area that causes definite discomfort

- **VISIA CR Imaging Procedures**

Clinic personnel ensured subjects had a clean face with no makeup and subjects removed any jewelry from the area to be photographed. Subjects were provided with a black or gray matte headband to keep hair away from the face and a black or gray matte cloth was draped over the subjects' clothing. Subjects were instructed to adopt neutral, nonsmiling expressions with their eyes gently closed, and were carefully positioned for each photograph.

A total of 3 full-face digital images were taken of each subject's face (left, center, and right views) using the VISIA CR photo station (Canfield Imaging Systems, Fairfield, New Jersey) with a Canon Mark II digital SLR camera (Canon Incorporated, Tokyo, Japan) under the following lighting conditions: standard 1 (visible [bright]), standard 2 (visible), and cross-polarized.

Each subject was provided with a preweighed unit of the test material [redacted] (Formula #52-33 / Lot #L3HA8142152)]. The following usage instructions were explained by clinic personnel:

Usage Instructions:

- Apply the test material according to the following instructions:
  This product should be applied nightly.
  1. Remove makeup and wash your face before using the product. The eye area (including brows, eyelids and lashes) should be completely clean and dry.
  2. Apply the product first to your lashes. Apply the product only along the upper lash line of both eyes. Dip the brush once per eye and wipe off any excess product from the brush before applying.
  3. Gently wipe off any excess serum from your eyelid or lashes. Do not wash your face or eyes after applying.
  4. Apply to your brows following the shape of your brows. Dip the brush once per eye and wipe off any excess product from the brush before applying. Gently wipe off any excess serum from your eye area. Do not wash your face or eyes after applying.
  5. Wait about 90 seconds until the product has dried before going to sleep or applying other products around your eye. Make sure to avoid eyelids, brows and lashes when applying any product to the eye area, including eye cream.

PROCEDURES AND METHODS (continued)

Usage Instructions (continued):

- If you get the serum in your eye, rinse with water. Some people may experience a mild tingling sensation after application. It should improve within a few minutes and disappear completely with continued use. Contact study personnel if tingling, irritation and/or redness persist.

Subjects were provided with a calendar of study visits, written usage/study instructions, and a daily diary to record product application times and comments.

Subjects returned to the clinic for visit 2 (week 1), visit 3 (week 2), visit 4 (week 3), visit 5 (week 4), visit 6 (week 5), visit 7 (week 6), visit 8 (week 7), and visit 9 (week 8). Subjects participated in the following procedures at each visit (unless otherwise indicated):

- Clinic personnel recorded concomitant medications and questioned subjects regarding changes in their health. AEs were recorded if applicable.
- Daily diaries were collected and reviewed for compliance. New diaries were distributed at each interim visit and completed diaries were retained by the clinic.
- Test material units were collected and visually inspected and weighed for compliance. Test material units were returned to subjects or new units were distributed as needed at each interim visit and retained by the clinic at study completion.
- Subjects acclimated for at least 15 minutes and then participated in the following procedures as previously described:
  - Cutaneous tolerance evaluation
  - Visual acuity test (at weeks 4 and 8 only)
  - Slit-lamp ophthalmological examination
  - VISIA CR imaging (at weeks 2, 4, 6, and 8 only)
- At weeks 2, 4, 6, and 8, subjects also participated in the following procedures:

  - **Self-assessment Questionnaires**
    Subjects completed Sponsor-provided self-assessment questionnaires analyzing the appearance of their lashes and brows and rating the treatment effects/product performance.

  - **Photo Comparison Grading of Images**
    A clinical grader compared VISIA CR images from each visit to images from baseline. The grader assessed each subject's lashes and brows separately for the following parameters (left and right sides were graded separately):
    - Longer-looking
    - Darker-looking
    - Fuller-looking

    The following scale was used:

Score	Description
-4	Very marked worsening
-3	Marked worsening
-2	Moderate worsening
-1	Minimal worsening
0	No change
1	Minimal improvement
2	Moderate improvement
3	Marked improvement
4	Very marked improvement

BIOSTATISTICS AND DATA MANAGEMENT

The PP population was the primary population for all statistical analyses testing. The PP population included all subjects who received treatment and completed the study in general accordance with the protocol.

For visual acuity test and slit-lamp ophthalmological examination, the data was analyzed separately for each eye as well as averaged for both eyes. The data from the left and right eyes and the average of both eyes were forwarded to the Sponsor as raw data.

For applicable evaluation parameters, a descriptive statistical summary is provided, including the number of observations (N), mean, median, standard deviation, minimum, and maximum at all time points.

Mean of the change from baseline (defined as the post-baseline value minus the baseline value) was estimated at each applicable post-baseline time point. The null hypothesis that the mean change from baseline is zero was tested using a Wilcoxon signed rank test.

Percent mean change from baseline and percent of subjects showing improvement or worsening were calculated using the following formulas:

$$\text{Percent mean change from baseline} = \frac{(\text{visit mean score} - \text{baseline mean score}) \times 100}{\text{baseline mean score}}$$

$$\text{Percent of subjects improved/worsened} = \frac{(\text{number of subjects improved/worsened from baseline}) \times 100}{\text{total number of subjects}}$$

Descriptive statistics and change from baseline statistics are presented in Table 6 through Table 8 for visual acuity test (including frequency of responses for eyewear), Table 9 through Table 11 for slit-lamp ophthalmological evaluation (including frequency of responses for contact lens), and Table 12 and Table 13 for cutaneous tolerance evaluation.

[redacted]

All statistical tests were 2-sided at significance level alpha=0.05. *P* values are reported to 3 decimal places (0.000). No multiple testing corrections were considered in the study. Statistical analyses were performed using SAS software version 9.4 series (SAS Statistical Institute).

Clinical grading scores were recorded using the Stephens electronic data capture (EDC) system, which is a computerized system designed for the collection of clinical data in electronic format. The 3 major aspects of EDC are a graphical user interface for data entry, a validation component to check for user data, and a reporting tool for analysis of the collected data. The Stephens EDC is compliant with Food and Drug Administration (FDA) regulations, namely the FDA's 21 CFR Part 11 regulation "Electronic Records; Electronic Signatures," which regulates the use of EDC in trials. Content validation procedures were performed to ensure adequate coverage of critical EDC system features.

The self-assessment questionnaires were completed by subjects electronically using HIPAA-compliant SurveyMonkey online survey software.

Images were forwarded to the Sponsor according to Stephens SOPs and any protocol specifications.

Note regarding n-values:
Refer to Protocol Deviations for data missing from statistical analyses.

QUALITY ASSURANCE

All clinical research studies performed by Thomas J. Stephens & Associates, Inc. are conducted in accordance with federal regulations and Good Clinical Practice guidelines. Stephens independent Quality Assurance Unit monitored the study conduct and audited the study documents, data, and clinical study report. All data and supporting documentation are accurate, complete, and in compliance with the requirements of the protocol and Stephens SOPs.

Data review and analyses were performed by an independent data committee, consisting of selected representatives from clinical services, quality assurance unit, and statistics department of Stephens. When requested, it was the responsibility of the independent data committee to send any interim data to the Sponsor.

MAINTENANCE OF RECORDS

All original records (including the study protocol, source documents, ICFs, screening/enrollment log, and any other records or forms used in this study) and a copy of the final report will be retained on file in the Thomas J. Stephens & Associates, Inc. archives for 2 years from the final report issuance date. When the archive time has expired, the study files will either be sent to the Sponsor at the Sponsor's expense or destroyed.

RESULTS AND CONCLUSIONS

Visual Acuity Test (Table 7)
Analysis of the visual acuity test showed no statistically significant changes from baseline in scores at weeks 4 or 8 for the left eye, right eye, or average of both eyes.

Slit-Lamp Ophthalmological Examination (Table 10)
Analysis of the slit-lamp ophthalmological examination showed a statistically significant increase (worsening) in fluorescein staining (the level of brightness and the percent of the eye that the stain covers) at week 1 for the right eye when compared with baseline. Although the increase was statistically significant, the clinical significance of a 0.5 increase (which was seen in 8 of the 10 subjects who had increase in scores between baseline and week 1) is negligible and not clinically relevant especially in the absence of corresponding subject complaints of dryness, burning, stinging or tingling. If the increase in stain were caused by the product itself, the Study Ophthalmologist would expect it to have been bilateral and to have continued, if not increased, throughout the course of the study.

There were no statistically significant changes from baseline in scores for bulbar conjunctival irritation (hyperemia, edema, erosions, and follicles), tarsal conjunctival irritation (hyperemia, edema, and follicles), lacrimation, or contact lens deposits (if applicable) at any post-baseline time point (weeks 1, 2, 3, 4, 5, 6, 7, and 8) for the left eye, right eye, or average of both eyes.

Additionally, there were no statistically significant changes from baseline in scores for subjective sensations (burning/stinging, itching, foreign body sensation, and soreness) at any post-baseline time point (weeks 1, 2, 3, 4, 5, 6, 7, and 8) for the left eye, right eye, or average of both eyes.

Cutaneous Tolerance Evaluation (Table 13)
Analysis of the cutaneous tolerance evaluation showed no statistically significant changes from baseline for any parameter (erythema, dryness, edema, burning, stinging, itching, tightness, and tingling) at any post-baseline time point (weeks 1, 2, 3, 4, 5, 6, 7, and 8) for the left eye, right eye, or eyebrows.

[redacted]

[redacted]

RESULTS AND CONCLUSIONS (continued)

Overall Conclusions

Overall results from this single-center clinical trial indicate that the Sponsor's eyelash conditioning serum [redacted] (Formula #52-33 / Lot #L3HA8142152)] did not cause any statistically significant worsening in visual acuity test or slit-lamp ophthalmological examination scores including subjective sensations [except fluorescein staining at week 1 on the right eye] when used over the course of 8 weeks by women in general good health, including those with self-perceived sensitive eyes, contact lens wearers, and regular users of mascara. Additionally, there was no statistically significant worsening in cutaneous tolerance evaluation scores for the eyes or eyebrows. Photo comparisons also identified significant improvement in lashes and eyebrows looking longer/darker/fuller.

A total of 4 subjects experienced AEs determined to be related to the test material; 2 subjects experienced mild eye pain, dry eye, and/or ocular hyperaemia **possibly** related to the test material; 1 subject with self-perceived sensitive eyes experienced moderate eye pain and eye irritation **probably** related to the test material; and 1 subject experienced mild ocular hyperaemia and erythema of the eyelids, and moderate eye irritation **definitely** related to the test material.

Results of the visual acuity test showed no statistically significant changes from baseline in scores at weeks 4 or 8 for the left eye, right eye, or average of both eyes.

Results of the slit-lamp ophthalmological examination showed no statistically significant changes from baseline in scores for bulbar conjunctival irritation (hyperemia, edema, erosions, and follicles), tarsal conjunctival irritation (hyperemia, edema, and follicles), lacrimation, contact lens deposits (if applicable), or any subjective sensations (burning/stinging, itching, foreign body sensation, and soreness) at any post-baseline time point (weeks 1, 2, 3, 4, 5, 6, 7, and 8) for the left eye, right eye, or average of both eyes. There was a statistically significant worsening in fluorescein staining (the level of brightness and the percent of the eye that the stain covers) at week 1 for the right eye when compared with baseline. The worsening was transient, as there were no statistically significant changes from baseline at weeks 2 through 8 for the level of brightness and the percent of the eye that the stain covers, and not clinically relevant, as the increase was very small and only in the right eye.

Results of the cutaneous tolerance evaluation showed no statistically significant changes from baseline for erythema, dryness, edema, burning, stinging, itching, tightness, or tingling at any post-baseline time point for the left eye, right eye, or eyebrows.

REFERENCES

1. Yoelin S, Wu J, Somogyi C, Beddingfield FC III. Inter-rater and intra-rater reliability of the Global Eyelash Assessment scale for assessment of overall eyelash prominence. Poster presented at: 33rd Annual Hawaii Dermatology Seminar of the Skin Disease Education Foundation; February 7-13, 2009; Maui, HI.

REPORT APPROVAL

Report approved by:

THOMAS J. STEPHENS & ASSOCIATES, INC.

APPENDICES

I. Safety/Tolerability Data Results Tables
II. Efficacy Data Results Tables
III. Questionnaire Data Results Tables
IV. Raw Data
V. Protocol, Protocol Deviation Log, Note to File for Biostatistics and Data Management
VI. Sample Forms
    Informed Consent Form
    Eligibility and Health Questionnaire
    Screening Form
    Clinical Grading Records
    Questionnaires
    Daily Diary
VII. Screening/Enrollment Log
VIII. Adverse Event Forms

Safety Assessment:

Isopropyl Cloprostenate

Using Available Data + QSAR Surrogates

Data Submission

to

Cosmetic Ingredient Review

May 16, 2024

By

Nick Skoulis, PhD
Senior Consulting Toxicologist
Steptoe LLP

Table of Contents

ABSTRACT

The safety of isopropyl cloprostenate (IPC) for use in cosmetic lash serums at levels of 0.0044% and 0.005% was previously reviewed by the Cosmetic Ingredient Review Journal (CIR), and data gaps were identified. As a result, CIR could not provide a definitive safety assessment for the use of IPC in cosmetic lash serum products and issued an Insufficient Data Announcement (IDA) on December 8, 2023. This submission provides additional data to address the requests in the IDA, as well as additional read-across analysis for similar compounds, to support a determination that the use of IPC in cosmetic lash serums at a level of 0.0075% may be considered safe to a reasonable certainty and not injurious to users.

INTRODUCTION

This report provides a comprehensive summary and data to support a determination of the safety of IPC for use in cosmetic lash serums at a 0.0075% use concentration, and is respectfully submitted in response to the CIR Expert Panel's IDA dated December 8, 2023. CIR already carried out an extensive review of the toxicology and safety assessment for IPC in a serum lash formulation containing 0.0044% and 0.005% IPC. Accordingly, this submission is intended to supplement that earlier evaluation, and to provide additional information to support a safety determination for IPC at the slightly higher use concentration of 0.0075%.

Previous quantitative structural assessment relationship (QSAR) analysis for IPC using Organization for Economic Cooperation and Development (OECD) Toolbox and ChemMines to assess the similarity of common pharmaceutical prostaglandins identified travoprost as a structurally similar compound. However, due to the presence of three fluorines in the travoprost structure, and on suspicion that might contribute to travoprost being slightly more toxic than IPC, the prostaglandin latanoprost was also chosen as a similar compound. Both travoprost and latanoprost have been favorably reviewed by the US Food and Drug Administration (FDA) and have robust data packages. In addition, the European Commission's (EC) Scientific Committee on Consumer Safety (SCCS) utilized cloprostenol in its review of the safety of IPC, as cloprostenol is a metabolite of IPC (SCCS, 2022).[1]

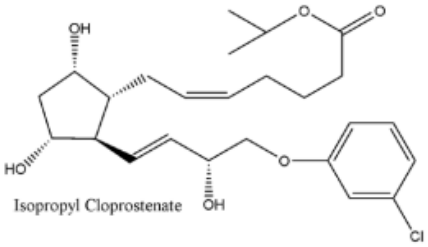
Accordingly, this report relies on both surrogate chemicals for read-across to fill in any potential data gaps, and additionally addresses the concerns presented in the SCCS Opinion on "Prostaglandins and Prostaglandin-Analogues used in Cosmetic Products." This includes addressing any data gaps and potential concerns for carcinogenicity, reproductive/developmental toxicity, and dermal sensitization, as well as positive response in an *in vitro* human lymphocyte assay. In addition, data are presented on similar formulations for cosmetic applications that contain IPC at the slightly greater amount (0.0075%, a small increase of 0.0025%), which further demonstrate that IPC produces similar, if not identical, results to those toxicological studies already presented to CIR on IPC.

[1] Scientific Committee on Consumer Safety (SCCS). Opinion: On Prostaglandin-Analogues Used in Cosmetic Products. SCCS/1635/21; adopted on February 3, 2022.

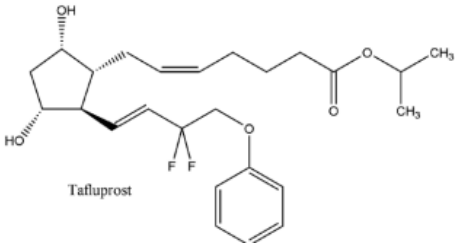
This approach provides a robust dossier which includes, but is not limited to, repeat topically applied toxicology studies, developmental and reproductive toxicity, both *in vitro* an *in vivo* mutagenicity testing, and full oncogenicity chronic bioassays. This approach supports a demonstration of safety for the use of IPC in cosmetic lash serum products by showing that products containing up to 0.0075% IPC are not irritating to the eyes or skin, and do not induce dermal sensitization. IPC is not genotoxic and, based on the oncogenicity studies of structurally similar prostaglandins, is not considered to be carcinogenic.

On these bases, we respectfully submit that the data discussed herein, together with the data and information previously reviewed by the Expert Panel for Cosmetic Ingredient Safety, support a demonstration that the use of IPC in lash serums at a level of 0.0075% may be considered safe to a reasonable certainty, and is not injurious to users.

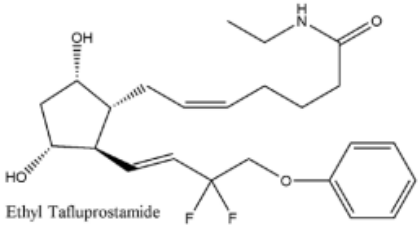
COMPOUNDS REVIEWED BY COSMETIC INGREDIENT REVIEW AT 167TH MEETING



Isopropyl Cloprostenate



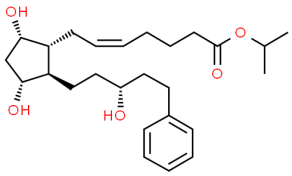
Tafluprost



Ethyl Tafluprostamide

Common Pharmaceutical Prostaglandins

Latanoprost



Latanoprost is derived from Prostaglandin F2α (PGF2a). The substance was favorably reviewed by the FDA for use in the eye to treat certain forms of glaucoma. Lantaonprost is highly selective for the Prostaglandin F (FP) type of prostanoid receptors. It is absorbed through the cornea, where esterases hydrolyze this prodrug and change it into a biologically active acid form.

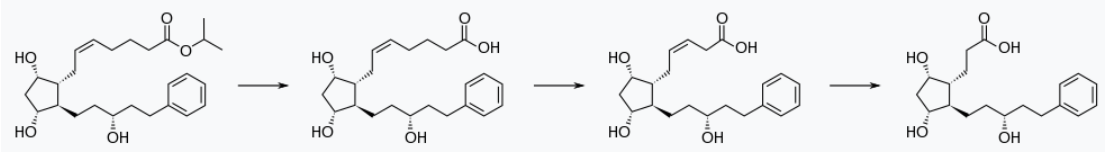
Latanoprost is currently used at a concentration of 0.005%.[2,3] Latanoprost has been shown to reach peak concentrations in the aqueous humor approximately 2 hours after instillation. If latanoprost is discontinued, Interocular Pressure (IOP) reportedly reaches pretreatment level after 14 days.[4]

Latanoprost produces less irritation to the ocular surface (causing hyperemia in 5% to 15% of subjects). The reported side effects of latanoprost include cystoid macular edema,[5] and benign changes in the iris' color.[6]

Because latanoprost breaks down more easily and becomes ineffective faster than the other prostaglandin analogs, it is sold in 5-mL bottles that have only 2.5 mL of fluid.

Due to the instability of latanoprost, it is recommended to be stored in the refrigerator. Latanoprost exhibits both thermal and photolytic instability. The concentration of Latanoprost when stored at elevated temperatures, i.e., 50°C, will decrease by 10% every 1.3 days; when exposed to natural sunlight this rate of decrease increases.[3]

Metabolism of Latanoprost[7]



Metabolism from left to right: latanoprost to latanoprost acid (active metabolite) to 1,2-dinorlatanoprost acid, and then to 1,2,3,4-tetranorlatanoprost acid.

[*] Xalatan (latanoprost ophthalmic solution) 0.005%. Package insert. 2006

---

2 Aung T, Wong HT, Yip CC, Leong JY, Chan YH, Chew PT (June 2000). "Comparison of the intraocular pressure-lowering effect of latanoprost and timolol in patients with chronic angle closure glaucoma: a preliminary study." *Ophthalmology*. **107** (6): 1178–1183. doi:10.1016/s0161-6420(00)00073-7. PMID 10857840.

3 Latanoprost Professional Drug Facts.

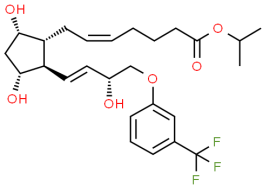
4 Xu L, Wang X, Wu M (2017). "Topical medication instillation techniques for glaucoma." *Cochrane Database Syst Rev*. **2017** (2): CD010520. doi:10.1002/14651858.CD010520.pub2. PMC 5419432. PMID 28218404.

5 "Drug Approval Package: Xelpros" *accessdata.fda.gov*. May 28, 2019. Retrieved December 27, 2023.

6 Johnstone MA, Albert DM (August 2002). "Prostaglandin-induced hair growth." *Survey of Ophthalmology*. **47** (Suppl 1): S185–S202. doi:10.1016/S0039-6257(02)00307-7. PMID 12204716.

7 Haberfeld H, ed. (2015). *Austria-Codex* (in German). Vienna: Österreichischer Apothekerverlag.

Travoprost

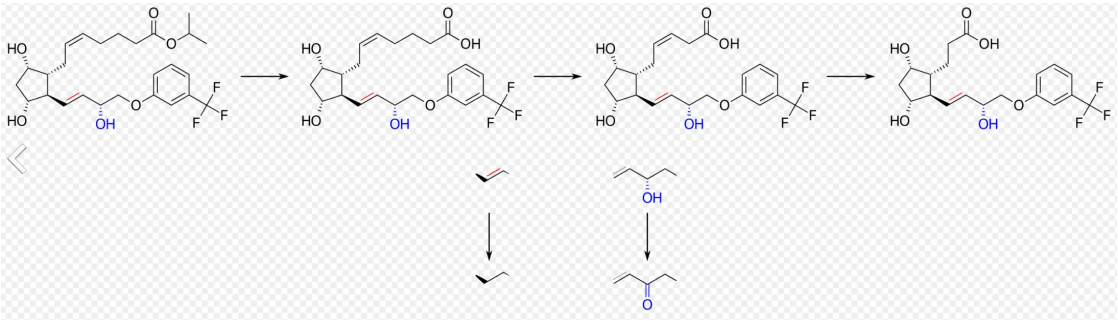


In 2001, the FDA approved travoprost, a highly selective FP prostanoid receptor agonist.

Metabolism of Travoprost[8, 9]

Travoprost is applied and absorbed through the cornea. In humans, peak plasma concentrations of travoprost free acid (25 pg/mL or less) were reached within 30 minutes following topical ocular administration and was rapidly eliminated. The side effects of travoprost include conjunctival hyperemia, and changes in the iris' color.

The figure below demonstrates the metabolism (from left to right): travoprost, travoprost acid (the active metabolite), 1,2-dinortravoprost acid, 1,2,3,4-tetranortravoprost acid. The reduction of the 13,14-double bond is identified in red and oxidation of the 15-hydroxy group in blue.



Travoprost, an isopropyl ester prodrug, is hydrolyzed by esterases in the cornea to its biologically active free acid. Systemically, travoprost free acid is metabolized to inactive metabolites via beta-oxidation of the a (carboxylic acid) chain to give the 1,2-dinor and 1,2,3,4-tetranor analogs, via oxidation of the 15-hydroxyl moiety, as well as via reduction of the 13,14-double bond.

---

8 Haberfeld, H, ed. (2015). *Austria-Codex* (in German). Vienna: Österreichischer Apothekerverlag. Travatan 40 Mikrogramm/ml Augentropfen.

9 NDA 21-257. Travatan™ – Travoprost Ophthalmic Solution (0.004%). Alcon Laboratories, Inc. Texas. U.S. Patent Nos. 5,631,287; 5,849,792; 5,889.052; and 6,011,062. © Alcon Laboratories, 2001.

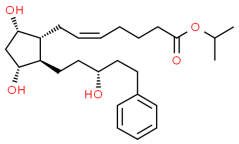
With regard to excretion, elimination of travoprost free acid from human plasma is rapid. Plasma levels are below the limit of quantitation (<10 pg/mL) within one hour following ocular instillation.

Adverse Events

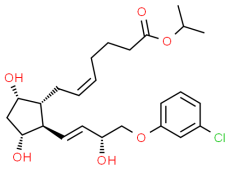
Local side effects are corneal changes, conjunctival hyperemia, and iris pigmentation, following daily use.[9]

Structural Activity Relationship

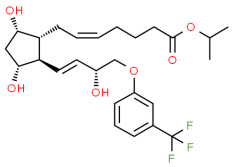
Structurally Similar prostaglandins favorably reviewed by the FDA compared to IPC are Latanoprost and Travoprost:



Latanoprost
CAS# 130209-82-4



Isopropyl Cloprostenate
CAS# 157283-66-4



Travoprost
CAS# 157283-68-6

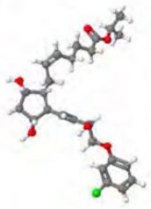
Both latanoprost and travoprost are, like other analogs of prostaglandin $F_{2\alpha}$, ester prodrugs of the free acid, which are agonists at the prostaglandin F receptor.[3,9] Prostaglandin F receptor (FP) is a receptor belonging to the prostaglandin (PG) group of receptors. FP binds to and mediates the biological actions of Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$).

ToxServices previously compared the structures of a few prostaglandin analogs using the ChemMine tools (ChemMine 2020) and the OECD Toolbox (OECD 2020). The Tanimoto coefficient, widely used for predictive accuracy for structural similarities (Chen & Reynolds 2002), also may be used in a structural activity relationship assessment.[10] The Tanimoto coefficient ranges from 0 to 1, with 0 being the least similar and 1 being the most similar. See the results below for comparing IPC to latanoprost and travoprost.[11]

---

[10] Chen, X. and Reynold, C.H. 2002. Performance of similarity measures in 2D fragment-based similarity searching: Comparison of structural descriptors and similarity coefficients. *J. Chem. Inform. Computer Sci* 42(6):1407-1414. https://doi.org/10.1021/cj025531g.

[11] ToxServices – Toxicology Risk Assessment Consulting. Report Title: Additional Dat for Consideration by CIR Pertaining to the Use of Isopropyl Cloprostenate (IPC) in Cosmetic Products. Redacted Company 2 (Markets Cosmetic Lash Serum at 0.005% IPC) Data Submission October 17, 2023.

	Isopropyl Cloprostenate	Latanoprost	Travoprost
SMILES	CC(C)OC(=O)CCC\C=C/C[C@H]1[C@@H](O)C[C@@H](O)[C@@H]1\C=C\[C@@H](O)COc2cccc(Cl)c2	CC(C)OC(=O)CCC\C=C/C[C@H]1[C@@H](O)C[C@@H](O)[C@@H]1CC[C@@H](O)CCc2ccccc2	CC(C)OC(=O)CCC\C=C/C[C@H]1[C@@H](O)C[C@@H](O)[C@@H]1\C=C\[C@@H](O)COc2cccc(c2)C(F)(F)F
Isopropyl Cloprostenate		**ChemMine** **AP Tanimoto: 0.623** **MCS Tanimoto: 0.465** **MCS Size: 20** **MCS Min: 0.645** **MCS Max: 0.625** **OECD Toolbox** **Tanimoto (Jaccard): 0.537**	**ChemMine** **AP Tanimoto: 0.742** **MCS Tanimoto: 0.861** **MCS Size: 31** **MCS Min: 0.969** **MCS Max: 0.886** **OECD Toolbox** **Tanimoto (Jaccard): 0.811**

Travoprost shows the greatest similarity to IPC, but we have included latanoprost as a potential surrogate as well. Based on the structures, the fluorides on the ring for travoprost would have a greater influence than the model might predict, and accordingly we believe that latanoprost and travoprost would bracket the toxicological effects of IPC. Specifically, latanoprost would be somewhat less toxic, and travoprost would be slightly more toxic, as compared to IPC, based on the fact that comparing fluorinated compounds on a molar basis tends to be more toxic than non-fluorinated similar compounds.[12] Below shows the structural relationship between the prostaglandin analogues and the surrogates that will be discussed in this review.

Figure 1. Structural formula of PGF2α, the pharmacologically active substances tafluprost, latanoprost, travoprost and bimatroprost, as well as other potential prostaglandin analogues identified in the CosIng database. The structural relationship between the substances is marked with arrows.

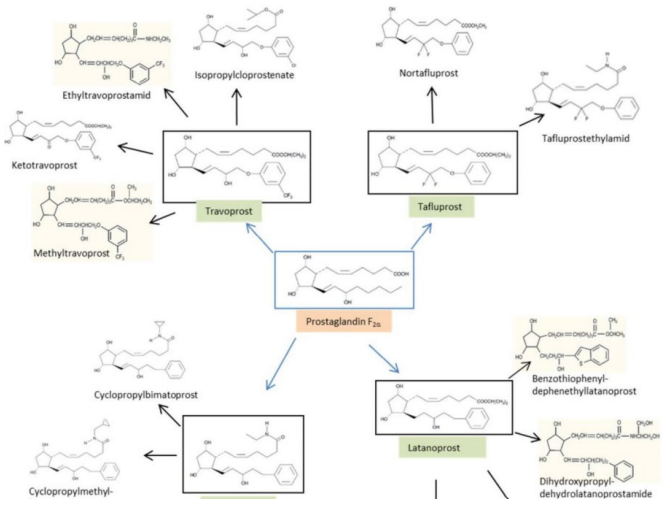


Diagram from the Federal Institute of Risk Assessment [Bundesinstitute für Risikobewertung] (cited in SCCS, 2021)[1]

[12] Pattison, F.L., Howell, W.C., and Woolford, R.G. Toxic Fluorine Compounds. DRB Report No. SW-81. Cdnsciencepub.com/doi/pdf/10.1139/v57-021 Can. J. Chem. 1956.

TOXICOLOGICAL EVALUATION

CIR was provided with analysis and data of IPC in advance of the December 4-5, 2023, meeting, which included information for: (1) acute and short-term toxicity and (2) male reproductive parameters assessed in mice, as well as QSAR analysis in relation to genotoxicity and carcinogenicity. Clinical Trials for IPC as well as case studies were also provided to CIR.[13] In addition, the SCCS evaluated cloprostenol for acute, subchronic, and reproductive toxicity. While the octanol/water coefficient varies between the two compounds, IPC through metabolism does generate cloprostenol.

As stated above, latanprost and travoprost are analogs of Prostogalndin $F_{2\alpha}$. PGF2α is upregulated during inflammation. Binding to its receptor activates G-protein coupled intracellular signal transduction pathways, resulting in increased intracellular calcium (Ricciotti and FitzGerald 2011).[14] In the eye, agonist-mediated activation of the PGF2α receptor ultimately causes relaxation of muscles controlling outflow of vitreous fluid and/or remodeling of the extracellular matrix via increased matrix metalloproteinase activity. The analogues may also upregulate endogenous prostaglandin production and/or may increase ocular blood flow (Ishida *et al*. 2006).[15]

Surrogate Toxicity & Pharmacodynamics: Travoprost & Latanoprost

Ocular Hypermia

Netland *et al*. found clinically significant changes in ocular hyperemia in 49.5% of subjects treated with travoprost 0.004%, in 27.6% of patients treated with latanoprost (0.005%), and in 14.0% of patients treated with timolol.[16] However, mean hyperemia score in all the treatments groups was less than 1 on a scale of 0–3, indicating that, on average, the majority of patients experienced none/trace to mild hyperemia. Hyperemia was evident since the first follow-up visit, at week 2.

Konstas *et al*. found no difference in ocular hyperemia incidence among patients treated with travoprost at a dose of 0.004% in the evening or in the morning. Respectively, hyperemia was encountered in 27% and in 33% of patients (P=0.6). In another study, the same authors

---

[13] Cosmetic Ingredient Review: Safety Assessment of Ethyl Tafluprostamide and isopropyl Cloprostenate as Used in Cosmetics. Draft Report for Panel Review. Release Date May 19, 2023; Panel Meeting Date June 12-13, 2023.

[14] Ricciottia, E., and GitzGerald, G.A. Prostaglandins and Inflammation. Arteriosclerosis, Thrombosis, and Vascular Biology (31)5:986-1000. 2011.

[15] Ishida, N., Odani-Kawabata, N., Shimazaki, A., and Hara, H. Protanoids in the Therapy of Glaucoma. Cardiovascular Drug Review (24)1:1-10, 2006.

[16] Honrubia, F., Garcia-Sanchez, J., Polo, V., Martinez de la Casa, J.M., and Soto, J. Conjunctival Hyperemia with the Use of Latanoprost versus other Prostaglandin Analogues in Patients with Ocular Hypertension of Glaucoma: A Meta-Analysis of Randomized Clinical Trials.Br. J. Ophthalmol. 93:316-321, 2009.

noted that conjunctival hyperemia was seen twice as often with travoprost (15 patients) as with latanoprost (six patients).[16]

Iris Pigmentation Changes

In a 12-month study, Netland *et al*. found iris pigmentation change in 3.1% of patients under therapy with travoprost 0.004% and in 5.2% of patients under therapy with latanoprost.[16]

As iris pigmentation appears to be a function of treatment duration, one might expect greater incidence of this side effect following long-term uses. Riva *et al*. evaluated the safety of travoprost treatment in a 5-year follow-up study, and found a cumulative incidence of iris darkening of 27.7%, which is comparable with the 33.4% rate reported in the 5-year study on latanoprost.[17]

Pharmacokinetics/Pharmacodynamics Absorption

Travoprost

Travoprost is absorbed through the cornea. In humans, peak plasma concentrations of travoprost free acid (25 pg/mL or less) were reached within 30 minutes following topical ocular administration and was rapidly eliminated.

With regard to metabolism, travoprost, an isopropyl ester prodrug, is hydrolyzed by esterases in the cornea to its biologically active free acid. Systemically, travoprost free acid is metabolized to inactive metabolites via beta-oxidation of the a(carboxylic acid) chain to give the 1,2-dinor and 1,2,3,4-tetranor analogs, via oxidation of the 15-hydroxyl moiety, as well as via reduction of the 13,14 double bond.

With regard to excretion, elimination of travoprost free acid from human plasma is rapid. Plasma levels are below the limit of quantitation (<10 pg/mL) within one hour following ocular instillation.[18]

Latanoprost

Latanoprost is rapidly absorbed in the cornea as an isopropyl ester prodrug and is then activated by the process of hydrolysis. Only a small amount of this drug is systemically absorbed. The maximal concentration of latanoprost in the systemic circulation is reached after 5 minutes and is measured to be 53 pg/mL, with the maximal concentration in the aqueous humor attained within 2 hours after administration, and has been estimated to be 15-30 ng/mL.[19]

---

17 Riva, I., Katsanos, A., Floriani, I., Biagioli, E., Konstas, A.G.P., Centofanti, M., and Quaranta, L. Long-Term 24-Hour Intraocular Pressure Control with Travoprost Monotherapy in Patients with Primary Open-Angle Glaucoma. J. Glacoma 23(8):535-540, 2014.

18 Alcon Laboratories. Travatan™ (Travoprost Ophthalmic Solution) 0.004%. NDA 21-257, U.S. Patent No. 5,631,287; 5,849,792; 5,889,052; and 6,011,062. 2001.

19 Pfizer. Xalatan Package Insert. Zalantan® – Latanoprost Ophthalmic Solution 0.005% (50 µg/mL). NDA 20-597/S-044; Reference ID: 3100250. August 2011.

The volume of distribution of latanoprost is 0.16 ± 0.02 L/kg. The activated acid form of latanoprost can be measured in aqueous humor in the initial 4 hours post-administration, and it is measured in the plasma only for 1 hour following ophthalmic administration. This drug is more lipophilic than its parent prostaglandin and easily penetrates the cornea. It has been shown to cross the placenta in rats.[19]

With regard to excretion, after hepatic beta-oxidation, the metabolites of latanoprost are primarily found to be excreted by the kidneys. About 88% of the latanoprost dose is recovered in the urine after topical administration. About 15% of a dose is reported to be excreted in the feces.[19]

With regard to half-life, the elimination half-life of latanoprost from the plasma is about 17 minutes. The elimination half-life of latanoprost from the eye is estimated at 2–3 hours.[19]

Acute Toxicity

No acute Oral Toxicity Studies for IPC could be located.

Latanoprost Oral LD_{50} is >20 mg/kg.[20]

Cloprostenol Oral LD_{50} is >25 mg/kg.[1]

Acute Toxicity Studies: Parenteral Isopropyl Cloprostenate

White albino Swiss mice (20/group; sex not stated) were administered a single dose of IPC (50, 75, or 100 mg/kg bw; dissolved in 1:19 dimethyl sulfoxide and water) via intraperitoneal injection, and observed for 14 d.[11] Two control groups were treated with physiological solution or dimethyl sulfoxide and water. No adverse effects regarding clinical parameters, mortality, or body weight were observed.

Evaluation of Conjunctival Hyperemia – Isopropyl Cloprostenate

Conjunctival hyperemia was evaluated in New Zealand albino rabbits.[13] The dose estimated to produce conjunctival hyperemia in 15% of the tested rabbits over a 4 h period was 0.3 µg. No other details were provided for this study.

The Hen's Egg Test (HET-CAM) in vitro test was utilized to evaluate the potential for IPC to induce irritation to the eye and surrounding mucosal membranes. A lash/brow formulation containing 0.0075% IPC was evaluated in the HET-CAM tests and scored at 0.5, 2,

[20] Reviewer: Rivera, M.I. Addendum to Pharmacology/Toxicology NDA Review and Evaluation. Center for Drug Evaluation and Research – Non-Clinical Reviews Application No. 206-185. Product Zelpros (Latanoprost) 0.005% Ophthalmic. Applicant – Sun Pharma Advanced Research Co., LTD. March 15, 2015.

and 5 minutes that provided a total average score of 1.75 and was classified as practically non-irritating.[21]

An EpiOcular eye irritation Test was carried out with eyelash serum containing 0.0075% IPC following OECD Guideline No. 492. Fifty µl of test article was applied to EpiOcular tissues in duplicate, with both positive and negative controls run in duplicate and concurrently. The results of the test demonstrated that the eyelash serum with 0.0075% was classified as a non-irritant.[22]

Dermal Irritation and Sensitization Studies – Isopropyl Cloprostenate

Human Repeat Insult Patch Tests (HRIPTs) were also performed using eyelash serums containing IPC (0.0044% and 0.005%; tested neat; n = 50-56).[14-17] The majority of assays were performed under semi-occlusive conditions. The serums (including IPC) tested were considered to be non-irritating and non-sensitizing in all assays.

Another HRIPT study evaluating an eyelash serum with 0.0075% IPC was carried out in 102 subjects, 23 males and 79 female subjects. Test material was evaluated under semi-occlusive conditions. Under the conditions of the test, the serum containing 0.0075% IPC at challenge the results indicated no potential for dermal sensitization and was not a skin irritant.[23]

Two additional HRIPT studies with the eyelash serum with 0.0075% of IPC, one study evaluated 58 subjects[24] and the other study had 54 subjects.[25] Both studies applied 20-50 mg of the product applied to the intrascapular region of the back. Results showed no signs of dermal irritation nor any signs of dermal sensitization.

Short-Term Toxicity Studies

Isopropyl Cloprostenate

Hematological evaluations were performed on white Wistar rats (10/group; sex not stated) treated with IP (15 mg/kg bw/d) for 7-days via intraperitoneal injection.[13] Control groups

---

[21] Nitka, S. The Hen's Egg Test – Utilizing the Chorioallantoic Membrane (HET-CAM); Lash-Brow Serum. Consumer Product Testing Co. Fairfield, New Jersey, Experiment Reference No. V15-2816. June 25, 2015.

[22] Troese, M. EpiOcular Eye Irritation Test (EIT). MB Research Laboratory, Spinnerstown, PA. MB Research Project No. 20-27939.19, May 11, 2020.

[23] Shoshani, L. 100 Subject Human Repeat Insult Patch Test for Skin Irritation and Skin Sensitization Evaluation. BioScreen Testing Services, Inc. Torrance, CA. Laboroatory Study No. 21-527A & 21-528A. September 15, 2021.

[24] Yazzie, B. 50 Subject Human Repeat Insult Patch Test for Skin Irritation and Skin Sensitization Evaluation. BioScreen Testing Services, Inc. Torrance, CA. BioScreen Study No. 15-404A. July 2015.

[25] Yazzie, B. 50 Subject Human Repeat Insult Patch Test for Skin Irritation and Skin Sensitization Evaluation. BioScreen Testing Services, Inc. Torrance, CA. BioScreen Study No. 18-501A. February 2018.

received a solution of dimethyl sulfoxide and water. Parameters evaluated include red blood cell count, hemoglobin, hematocrit, and red/white cell indices. Two hours after the last administration, animals were euthanized and blood was examined. Results were similar among control and treated groups.

Latanoprost

Latanoprost was evaluated in a 30-day toxicity study where the compound was administered 4 times a day to New Zealand White rabbits at 30 µL once a day of 0.005% latanoprost/eye; 30 µL/eye twice a day of a 0.005% latanoprost solution; and 30 µL/eye 4 times a day of a 0.005% latanoprost solution. Results showed no adverse findings in the eyes nor histopathologic changes. One male at week 5 was observed to have conjunctival redness (score of 2.0). No systemic effects were observed based on clinical signs, bodyweights, hematological evaluations, clinical chemistry, urinalysis, gross pathology, selected organ weights, and histopathology. NOTE: levels of latanoprost free acid was below the levels of quantitation (50.3 pg/mL) with the exception of 2 mid-dosed males and 3 high-dosed males at the 0.5 hr and 1 hr timepoints on the first day of dosing. Concentrations were 89.7 – 192.8 pg/mL and 54.3 – 59.5 pg/mL at the 0.5 hr and1 hr timepoints, respectively.

The NOAEL was 20 µg of latanoprost based on four 30 µL/eye of a 0.005% ophthalmic solution.[26]

Travoprost

Travoprost was evaluated in New Zealand White rabbits in a 13-week toxicity study to evaluate ocular irritation as well as any systemic toxicity. Fifty µL/eye was administered 3-times per day of a 0.004% travoprost ophthalmic solution (free of the preservative benzalkonium chloride, BAC). Males were exposed for 91-days and females were exposed for 92-days. Results showed that one animal in week-9 shoed moderate conjunctival discharge with no remarkable indirect ophthalmoscopy findings being noted at the week-13 examinations. No toxicologically significant findings were noted in clinical chemistries, nor in the hematological and coagulation parameters in any of the treated animals. There was a slight decrease in bodyweights, but this finding failed to achieve statistical significance. Necropsy of the animals showed no findings that were considered to be test-article related. Histopathology was carried out in the treated eye of the animals as well as in selected organs, it was concluded that there were no test-article related lesion in ocular nor non-ocular tissues.

The NOAEL was 12 µg of travoprost administered 3-times at 50 µL per dosing for 90-days.[27]

---

[26] Reviewer Rivera, M. US FDA Center for Drug Evaluation and Research, Application No. 206185Orig1s000; Non-Clinical Review(s) Addendum to Pharmacology/Toxicology NDA Review and Evaluation Xelpros (Latanoprost) 0.005% Ophthalmic Solution. CDER Stamp Date January 31, 2014.

[27] Reviewer Chen, Z. US FDA Center for Drug Evaluation and Research, Application No. 21-994 Pharmacology Toxicology Review: Travatan® Z. 11/21/2005.

Developmental & Reproductive Toxicity Studies

Isopropyl Cloprostenate

The effect of IPC on the apoptosis of male mice (20/group; strain not stated) and Wistar rat (20/group) testicular cells was evaluated in a 28-d study. 10 intraperitoneal injections of the test substance were given to mice in a dose of 25 µg/kg bw/d, and to rats in doses of either 25 or 100 µg/kg bw/d. Control groups of mice and rats were left untreated. Animals were killed at different time intervals (7, 14, and 28 days of treatment), and histological examinations of the gonads were performed. Normal structures of the testicular cells were observed in control groups.

In rats treated with IPC100 µg/kg bw/d, enlarged blood vessels were noted. Blood vessel diameter increased in a time-dependent manner.

This effect was also noted in rats treated with 25 µg/kg bw/d; however, the increase in blood vessel diameter was smaller. After 14 and 28 days of treatment, hyaline-like material was observed in the interstitial space surrounding the seminiferous tubules in rats treated with 100 µg/kg bw/d. Also observed in this group was accumulation of polymorphonuclear neutrophils and macrophages, reduced spermatozoa, affected spermatogenesis, and nuclear condensation of the testicular cells. Macrophages, decreased spermatozoa, and affected spermatogenesis were observed in treated mice. A similar study was performed in male mice (12 mice/group; strain of mice not specified).[1] Mice were treated with IPC (25 µg/kg bw/d) for 28 days via intraperitoneal injection. A control group of mice was left untreated. After 7, 14, or 28 days, animals were killed and effects on the gonads were examined.

Results revealed swollen endothelial cells, macrophages with residual bodies, a large number of fibroblasts in interstices, lysosome-like dense bodies in the cytoplasm of Sertoli cells, clumped erythrocytes in capillaries, spermatocytes with condensed cytoplasm, and nuclei with a high chromatin condensation. (SCCS, 2022).[1]

While this study suggests that there is a potential for IPC to have an effect on sperm numbers, it should be pointed out that the administration of IPC via intraperitoneal injections is not a recommended route of administration because it places the chemical of interest in close proximity to the reproductive organs and the resulting dynamics of the dose and concentration would not be reflective of oral or dermal exposure; thus, the biological relevance of these results is questionable.

Cloprostenol

SCCS (2022)[1,28] summarized a 3-generation study carried out in rats, where oral administration of doses of 0, 10, 15, 20, and 40 µg of cloprostenol/kg bw did not induce effects on reproductive performance of the animals at any of the does tested. The only effects seen were

[28] European Medicines Agency. Committee for Medicinal Products for Veterinary Use – Cloprostenol and R-Cloprostenol. Summary Report. EMEA/MRL/898/04-Final. May 2004.

the slight reduction in neonatal viability attributable to the prematurity of the offspring. A No Observed Effect Level (NOEL) of 15 µg/kg bw/day for cloprostenol was reported.

In a series of reproductive studies performed with cloprostenol, it was shown that the sensitivity of the rat to termination of pregnancy resulting from luteolysis varies depending on the point in pregnancy when the compound is administered. The oral dose of 25 µg/kg bw of cloprostenol did not terminate pregnancy; the most sensitive period to luteolytic action of cloprostenol was just prior to the parturition.

No teratogenic properties of cloprostenol were reported in the two teratogenicity studies performed either in rats after oral administration of 0, 10, 25, 50, and 100 µg/kg bw/day, or in rabbits after subcutaneous administration of 0, 0.025, 0.075, and 0.250 µg/kg bw/day.[1,28]

Travoprost

ToxServices previously summarized the reproductive and developmental toxicity for Travoprost,[11] as it was evaluated for reproductive and developmental toxicity in rats. Pregnant Sprague-Dawley rats were dosed at 0, 1, 3, and 10 µg/kg/day by intravenous injection on gestation days (GD) 6-17 and were sacrificed on GD 20. Maternal body weight gain and gravid uterine weight were reduced. The incidence of total litter resorptions was increased, as were the numbers of early and late resorptions. Premature delivery also was observed. The numbers of corpora lutea and implantations were reduced below historical control values, but there was no net effect on pre-implantation loss. Fetal viability and fetal body weights were also reduced. Lastly, the incidences of fetal external, visceral, and skeletal malformations and variations were increased.

The authors identified a NOAEL of 3 µg/kg/day for this study (FDA 2000).[29]

In another study in Sprague-Dawley rats, animals were dosed by s.c. injection at 0, 1, 3, and 10 µg/kg/day travoprost for 4 weeks prior to and through GD 13 (females) or 2 weeks prior to mating through GD 7 (males). Corpora lutea, implantations, fetal viability, estrous cyclicity, and sperm parameters were not affected by treatment. The authors reported an increase in early fetal resorptions at 10 µg/kg/day. The NOAEL for this study was 3 µg/kg/day (U.S. FDA 2000).

One developmental study in mice also was identified. Female CD-1(ICR)BR mice were given s.c. injections of 0, 0.1, 0.3, and 1 µg/kg travoprost on GD 6-16 and were sacrificed on GD 18. The incidences of early deliveries, litter loss, and total litter resorption as well as the number of early resorptions were increased, while the number of viable fetuses was reduced. No teratogenic effects were observed in the fetuses. Authors identified a NOAEL of 0.3 µg/kg/day for this study (FDA 2000).

---

[29] United States Food and Drug Administration (U.S. FDA). 2000. Pharmacology review of Travoprost Ophthalmic Solution (NDA 21-257). Available: https://www.accessdata.fda.gov/drugsatfda docs/nda/2001/21257 Travatan.cfm.

Latanoprost

In an embryofetal development toxicity study in rabbits, there was a significant increase (2.4 times the human dose) in the number of resorptions at the high dose of 464 mg/kg/day IV administered from gestation days 6 through 18. As a consequence, an increase in post-implantation loss and a decrease in live fetuses was observed at the same dose. In addition, fetal incidences of misaligned sternebrae and total skeletal variations were increased in the high-dose group and were considered statistically significant (4 and 2-times over controls, respectively ≤ 0.05). The NOAEL for fetuses was 215 mg/kg; and the NOAEL for dams was 464 mg/kg.

In a combined embryotoxicity and pre- and postnatal reproduction toxicity study in rats no external, visceral or skeletal abnormalities at doses of up to 464 mg/kg/day IV when dosing from gestation days 6 through 17. Extension of dosing from Day 6 of gestation to Day 21 of lactation did not show adverse effects on prenatal or postnatal development, nor was maternal function significantly affected at doses up to 464 mg/kg/day. The maternal and fetal NOAEL was 464 mg/kg/day.

The exposure margins based on mg/m^2 indicate there is no concern for reproductive or embryofetal effects after topical ocular administration of latanoprost ophthalmic 0.005% at the intended clinical dosing regimen.[30]

Exposure Margins for Induced Embryotoxicity

Species	NOAEL (mg/kg)	NOAEL (mg/m^2)	Exposure Margin*
Rabbits	215	2580	27,892
Rats	≥464	≥2784	≥30,097

*Human dose of one drop of 0.25% = 0.075 mg/eye (30 µL drop) = 0.15 mg/day (both eyes treated) = 0.0025 mg/kg (60 kg body weight) = 0.0925 mg/m^2

Genotoxicity Studies

Isopropyl Cloprostenate

A QSAR model and a statistical-based model of an Ames test on IPC predicted no genotoxicity.[1]

Travoprost

Travoprost, as part of the FDA (2001) review, was evaluated in the Ames assay, *in vivo* mouse micronucleus, and in a rat chromosomal aberration assay, and was found not to be genotoxic. Two mouse lymphoma assays were conducted; one study showed clear non-

[30] US FDA Center for Drug Evaluation and Research, Application No. 206185Orig1s000; Non-Clinical Review(s) Addendum to Pharmacology/Toxicology NDA Review and Evaluation Xelpros (Latanoprost) 0.005% Ophthalmic Solution. CDER Stamp Date January 31, 2014.

mutagenic results, while the second study was observed to produce a slight increase in the mutation frequency.

The weight of evidence would support the fact that travoprost would not be genotoxic, especially since the higher tier study, *in vivo* mouse micronucleus was determined to be non-genotoxic.[29]

Latanoprost

Latanoprost was found to be non-genotoxic in the Ames assay, mouse lymphoma, or in the mouse micronucleus assay. However, in the *in vitro* human lymphocytes study, chromosomal aberrations were observed.

The mouse micronucleus assay is an *in vivo* assay and supports the weight of evidence that latanoprost is non-genotoxic.[30]

Carcinogenicity Studies

Isopropyl Cloprostenate

An in-silico analysis of IPC was flagged for potential carcinogenicity with a reasonable model certainty, raising the concern that this ingredient may be a non-genotoxic carcinogen, though we do not expect this in-silico analysis to be correct, as discussed below.[1] The mechanism of action for prostaglandins to induce a carcinogenic response is believed to be through the PGE2 receptors, while IPC and the surrogate analogues interact through the PGF2α receptors. Chen *et al*. (2022) reported that in hepatocellular carcinoma, a common liver cancer with a high incidence rate globally, the cytokine PGE2 is shown to be overexpressed in various human malignancies, including hepatocellular carcinoma. PGE2 binds to EP receptors in the liver hepatocyte and influence s tumorigenesis and/or enhance tumor progression through multiple pathways. PGE2 can promote the proliferation and migration of liver cancer cells by affecting hepatocytes directly.[31]

Travoprost & Latanoprost

Latanoprost was found not to be carcinogenic in either mice or rats when administered via oral gavage at doses up to 170 µg/kg/day (a dose approximately 2800 times the maximum human dose), where mice were treated up to 20 months and rats up to 24 months. (FDA, NDA 2011).[32]

Travoprost was evaluated in two-year bioassays, in which both rats and mice were dosed with travoprost via subcutaneous injection at doses up to 100 micrograms/kg/day (2,500 times

---

[31] Chen, C., Guan, J., Gu, X., Chu, Q., and Zhu, H. Protaglandin E2 and Receptors: Insight into Tumorigenesis, Tumor Progression, and Treatment of Hepatocellular Carcinoma. Frontiers in Cell and Developmental Biology doi: 10.3389/fcell.2022.834859. March 10, 2022.

[32] US FDA NDA. Xalatan® – Latanoprost Ophthalmic Solution (0.005%). Pfizer, USA. Revised August 2011.

the recommended clinical dose), with the results revealing no evidence of carcinogenic effect. (Travoprost Product Monograph, 2019).[33]

Mechanism of Action

Chen *et al*. (2022) reported that in hepatocellular carcinoma, a common liver cancer with a high incidence rate globally, the cytokine PGE2 is shown to be overexpressed in various human malignancies, including hepatocellular carcinoma. PGE2 binds to EP receptors in hepatocyte carcinoma that influence tumorigenesis and/or enhance tumor progression through multiple pathways. PGE2 can promote the proliferation and migration of liver cancer cells by affecting hepatocytes directly.[28] However, IPC is a PGF2a cytokine and is not known to bind to the EP receptors. The in-silico assessment of IPC most likely mistakenly assigned a high certainty for carcinogenicity based on the fact that hepatocellular cancer is believed to be driven through prostaglandin receptors.

Other Relevant Studies

Pupil Constriction – Isopropyl Cloprostenate

The effect of IPC on the constriction of pupils was evaluated in cats.[13] Potency was expressed as an ED5 value which represents the dose estimated to produce a 5-unit area (mm*h) in a graph of the difference in pupil diameter in the dosed eye versus time (or median effective dose). The ED5 for PIC was determined to be 0.013 µg. No other details were provided in this study.

Ophthalmological In-Use Safety Evaluation – Isopropyl Cloprostenate

An eyelash serum containing 0.0075% was evaluated for safety following repetitive, daily use conditions. The product is designed moisturize and condition the eyelashes and eyebrows to enhance their appearance. On weeks 2, 4, 6, and 8 subjects were evaluated and compared to baseline. The subjects were assessed and graded the subject's eyelashes and eyebrows with the left and right sides were graded separately. At each timepoint the subjects were assessed for visual acuity at week 1 (baseline), and on weeks 4 and 8; slit-lamp examination by the Study Ophthalmologist performed on each subject's eyes and included grading for conjunctival irritation (hyperemia, edema, erosions, and follicles); lacrimation, contact lens deposits (if applicable), and fluorescein staining; and cutaneous tolerance evaluation that included objective irritation, and subjective irritation.

The overall conclusions from the study indicated that the eyelash serum containing 0.0075% IPC did not cause any statistically significant worsening in visual acuity test or slit-lamp examination scores that included subjective sensations when used over 8-weeks by women

[33] Product Monograph: Pr TRAVATAN® Z Travorpost Ophthalmic solution. Novartis Pharmaceuticals, Canada. August 2019.

in general good health. Furthermore, there was no statistically significant worsening in cutaneous tolerance evaluation scores for the eyes or eyebrows.[34]

Clinical Studies: Clinical Trial – Isopropyl Cloprostenate

The effect of an eyewash containing IPC (0.01%) in a phosphate buffer solution was evaluated in 23 patients.[1] The eye wash was applied to the eyes once daily for 3 months. Over the treatment period, no changes in visual acuity or papilla appearance were observed. Mild hyperemia of the bulbar conjunctiva was observed; however, this was reported to disappear after 2-3 days of treatment. No other adverse effects were observed.

Risk Assessment – Isopropyl Cloprostenate

ToxServices previously did a thorough review of the data and provided a margin of safety (MOS; calculated as the ratio between a point of departure (PoD)) and systemic exposure dosage (SED)) calculation on IPC.[11] The MOS was determined to be 2.5 (with an estimated combined SED of 0.00000084 mg/kg bw/d from eyelash and eyebrow products). In the calculation, the toxicological screening value (TSV) was used as the PoD; a detailed numerical value was not available due to confidentiality issue); thus, a MOS $\geq$ 1 was considered to be protective.

It is important to note that eyelash formulas are not free flowing liquids and are more like a viscous material that allows the formula to adhere to the eye lash and to allow homogenous distribution of IPC throughout the formulation. Guo *et al*. (2023)[35] reported the typical eyelid surface area to be 5.6 cm^2 and estimated daily application of eyelash formula is in the range from 4-10 mg and, for these purposes, we will use 7 mg as the median. Eyelash formula contact would be expected to be thin line along the bottom of the eyelid, roughly 2 cm x 0.2 cm (0.4 cm^2). Estimated dermal penetration of IPC is 10% for a free-flowing liquid, where the bioavailability would be reduced in a more viscous formulated product which would result in little to no material being absorbed. C

Pharmacokinetic data for travoprost and latanoprost reported concentrations in pg/ml, with peak plasma levels in humans is 25 pg/ml or less at 30 minutes following topical administration of one drop of 0.004% travoprost ophthalmic solution. Concentrations for IPC would be expected to be similar or less based on physical nature of the eyelash formulation and limited contact to the eyelid. Dermal absorption of IPC was estimated using a QSAR model. The estimated dermal absorption was determined to be 10% (based on a molecular weight of 476 g/mol and a log Kow of 5.15 for IPC). Assuming the maximum amount of eyelash product applied per application is 4 mg to 10 mg the amount of IPC applied per brush stroke would be

---

[34] Jiand, L., Stephens, M.T., and Acevedo, S. A Single-Center Clinical Study to Evaluate the Safety and Efficacy of [CONFIDENTIAL] when Used on Eyelashes and Eyebrows. Thomas J. Stephens & Associates, Inc., Stephens Study No. C18-D094; Sponsors Unpublished Study No. 2018TSA025. April 25, 2019.

[35] Guo.y., Rokohl, A.C., Fan, W., Theodosiou, R., Li, X., Lou, L. Gao, T., Lin, M., Yao, K., and Heindl, L.M. A Novel Standardized Approach for the 3D Evaluation of Upper Eyelid Area and Volume. Quant Imaging Med Surg 13(3):1686-1698, 2023.

0.0075% × 7.0 mg eyelash product (median concentration applied) = 0.5 µg. Thus, based on the estimated dermal absorption of 10%, the estimated amount of IPC that would be dermally absorbed from an eyelash product containing 0.0075% IPC would be 0.05 µg per use; and this is a conservative amount based on the physical nature of the eye las formula and minimal contact with the eyelid.

DATA GAP for ISOPROPYL CLOPROSTENATE

The Cosmetic Ingredient Review for IPC indicated that the following tests, highlighted in bold text, were needed to complete a safety assessment; we summarize below in italicized text why those data gaps are now addressed:

- **Dermal Irritation and Dermal Sensitization studies at 0.0075% IPC**

  IPC at 0.0075% was evaluated in 3 HRIPT where all three tests showed no evidence of dermal irritation or dermal sensitization in over 200 human subjects.

- **28-Day Dermal Toxicity Study with IPC**

  While no 28-day or greater repeat exposure studies could be located for IPC, the surrogate, latanoprost, has a 30-day topical ocular study in the rabbit evaluating ocular and systemic effects. In addition, the surrogate travoprost has a 13-week topical ocular study in the rabbit evaluating ocular and systemic effects.

- **Developmental & Reproductive Toxicity studies with IPC**

  Cloprostenol has a 3-generation reproduction/fertility study and an oral developmental toxicity study; travoprost has a developmental and reproductive toxicity studies; and latanoprost has a developmental and postnatal reproductive toxicity studies.

- **In vitro and in vivo genotoxicity studies looking for chromosomal aberrations with mammalian cell lines**

  Latanoprost has the following mutagenicity assays: Ames assay, mouse lymphoma, a mouse micronucleus assay, and an in vitro human lymphocytes study. Travoprost has the following genotoxicity studies: Ames assay, in vivo mouse micronucleus, a rat chromosomal aberration assay, and two mouse lymphoma assays; R-Cloprostenol has an Ames test, mouse lymphoma L5178Y/TK+/-, and an in vitro chromosomal aberration assay in human lymphocytes.

- **In-Silico Assessment of IPC, Oncogenicity Study**

  The SCCS reported on an in-silico assessment of IPC as a possible non-genotoxic carcinogen; however, it is important to note that hepatocellular carcinoma is a common liver cancer with approximately 750,000 cases annually on a global basis and PGE2, a pro-inflammatory cytokine, is over expressed in various human malignancies including hepatocellular carcinoma. PGE2 binds to EP receptors that have been shown to

influence tumorigenesis or tumor progression through multiple pathways. The treatment of hepatocellular carcinoma is designed through selective EP receptor antagonists that have shown to achieve beneficial outcomes. This strongly suggests that tumorigenesis and tumor progression is highly influenced through the EP receptors.

IPC, travoprost, and latanoprost all are considered F2α agonists, and do not interact with the EP receptors. To further support the conclusion that IPC is not carcinogenic, both Latanoprost and Travoprost were shown to be non-carcinogenic in chronic bioassays in both mice and rats.

OVERALL CONCLUSIONS – ISOPROPYL CLOPROSTENATE

According to 2023 Voluntary Cosmetic Registration Program (VCRP) data from FDA, IPC is used in three "other eye makeup preparation" formulations. However, according to data submitted by industry to CIR, two eyelash serums were determined to contain 0.0044% and 0.0048% IPC. An estimate of dermal absorption for IPC was determined to be 10%, according to modeling data.[15] This value was based on a molecular weight of 476 g/mol and a log Kow of 5.15.

An acute toxicity assay was performed in rats given IPC in dimethyl sulfoxide and water (up to 100 mg/kg bw) via intraperitoneal injection. No adverse effects were observed throughout the 14-d observation period. A hematological analysis was performed in rats given Isopropyl Cloprostenate (15 mg/kg bw/d), via intraperitoneal injection, for 7 d. No hematological abnormalities were observed.

Based on an in-silico analysis, the SCCS flagged IPC as potential reproductive/developmental toxicants. The effect of IPC (25 or 100 µg/kg bw/d) on gonads and testicular cells was evaluated in mice and rats. In these assays, animals were treated for 28 days, and sacrificed at different time intervals prior to evaluation. Time- and dose-dependent adverse effects (e.g., enlarged blood vessels, macrophages, reduced spermatozoa, reduced spermatogenesis, dense bodies in cytoplasm of Sertoli cells, clumped erythrocytes) were observed in treated animals. However, the route of administration of IPC in these assays was via i.p. injection, which is not an appropriate route of administration, as it concentrates the IPC in the vicinity of the target organ of interest and, at best, is significantly overly conservative given that, with oral or dermal exposures, such concentration would never be reached.

A QSAR model and a statistical-based model of an Ames test on IPC predicted no genotoxicity. Although being predicted to be non-genotoxic, the SCCS flagged Ethyl Isopropyl Cloprostenate for potential carcinogenicity based on an *in silico* analysis. However, it is important to note that hepatocellular carcinoma is a common liver cancer with approximately 750,000 cases annually on a global basis and PGE2, a pro-inflammatory cytokine, is over expressed in various human malignancies including hepatocellular carcinoma. PGE2 binds to EP receptors that has been shown to influence tumorigenesis or tumor progression through multiple pathways. The treatment of hepatocellular carcinoma is designed through selective EP receptor antagonists that have been shown to achieve beneficial outcomes. This strongly suggests that tumorigenesis and tumor progression is highly influenced through the EP receptors. To further

support the conclusion that IPC is not carcinogenic, both Latanoprost and Travoprost were shown to be non-carcinogenic in chronic bioassays in mice and rats.

We respectfully submit that the data and analysis presented in the draft safety assessment from CIR for IPC demonstrate that the increase in concentration (i.e., to 0.0075% IPC in the lash serum) is adequately addressed, based on the *in vitro* data from HET-CAM and the EpiOcular, and three HRIPT studies in over 200 human subjects with no indications of dermal irritation or dermal sensitization, which is consistent with the results from the testing of 0.005% IPC data. Based on the similarities in structure to lanoprost and travoprost, both of which have been favorably reviewed already by FDA for use in and around the eye, IPC use could possibly result in slight irritation of the upper eyelid from incidental contact with the eye where esterases could potentially act to form the acid of the active molecule. However, IPC would not be expected to be genotoxic or carcinogenic based on the strong evidence that tumorigenesis and progression of tumors appears to be through the EP receptors and IPC would not be expected to interact with the EP receptors. Furthermore, the one short-term study carried out with IPC that showed testicular effects is based on the administration of IPC via i.p. injection, which would result in a highly conservative approach, as i.p. injections result in placing a high concentration of the compound of interest in close proximity to the target tissue.

The directions for safe use provided in product labeling minimizes *the potential* for incidental contact below the waterline of the eye and is controlled so that such exposures would be infrequent to non-existent. Moreover, the pharmacokinetic data for both latanoprost and travoprost with multiple administrations directly to the eye results in maximal systemic levels measured in the pg/ml level, with a rapid elimination from the systemic circulation.

In summary, this review further supports ToxServices conclusions that a sufficient margin of safety exists for IPC, based on anticipated, controlled exposures and data reviewed for both surrogates latanoprost and travoprost. The localized effects, such as eye or skin irritation, or skin sensitization, are not expected based on the results from the three HRIPT studies evaluated IPC at 0.0075%, in vitro HET-CAM, or EpiOcular results. As noted previously, the physical properties of eyelash formulas are such that they are not a free-flowing liquid, and come in contact with eye rarely, with highly limited contact with the eyelid. These properties would result in concentrations in the systemic circulation to be at or below those levels reported for the surrogates travoprost and latanoprost.

Based on the use of the surrogates that have already been favorably reviewed by FDA, as well as the additional data summarized in this report, all data gaps have been filled for: repeated exposures, eye and skin irritation, developmental and reproductive toxicity, mutagenicity tests, and oncogenicity tests in two species. While the affinity coefficients have not been addressed for the receptors, the CIR cites a one-year study with IPC (0.005%) showing no signs of side effects reported, such as iris color change or other side effects.

On these bases, we respectfully submit that the use of IPC in lash serums at a level of 0.0075% may be considered safe to a reasonable certainty, and is not injurious to users.

LIST OF EXHIBITS

Exhibit No. Description

1 ..... Nitka, S. The Hen's Egg Test – Utilizing the Chorioallantoic Membrane (HET-CAM); Lash-Brow Serum. Consumer Product Testing Co. Fairfield, New Jersey, Experiment Reference No. V15-2816. June 25, 2015.

2 ..... Troese, M. EpiOcular Eye Irritation Test (EIT). MB Research Laboratory, Spinnerstown, PA. MB Research Project No. 20-27939.19, May 11, 2020.

3 ..... Shoshani, L. 100 Subject Human Repeat Insult Patch Test for Skin Irritation and Skin Sensitization Evaluation. BioScreen Testing Services, Inc. Torrance, CA. Laboratory Study No. 21-527A & 21-528A. September 15, 2021.

4 ..... Yazzie, B. 50 Subject Human Repeat Insult Patch Test for Skin Irritation and Skin Sensitization Evaluation. BioScreen Testing Services, Inc. Torrance, CA. BioScreen Study No. 15-404A. July 2015.

5 ..... Yazzie, B. 50 Subject Human Repeat Insult Patch Test for Skin Irritation and Skin Sensitization Evaluation. BioScreen Testing Services, Inc. Torrance, CA. BioScreen Study No. 18-501A. February 2018.

6 ..... Jiand, L., Stephens, M.T., and Acevedo, S. A Single-Center Clinical Study to Evaluate the Safety and Efficacy of [CONFIDENTIAL] when Used on Eyelashes and Eyebrows. Thomas J. Stephens & Associates, Inc., Stephens Study No. C18-D094; Sponsors Unpublished Study No. 2018TSA025. April 25, 2019.

Commitment & Credibility since 1976

Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Christina L. Burnett, MSES, Senior Scientific Analyst/Writer, CIR
Date: May 24, 2024
Subject: Wave 2 - Safety Assessment of 1,2,4-Trihydroxybenezene as Used in Cosmetics

Please find attached the comments provided by the Personal Care Products Council on the Draft Final Report on 1,2,4-Trihydroxybenzene (*PCPCcomments_Trihydroxybenzene_Wave2_062024)*. You can find the expert opinions mentioned in the comments on pdf pages 254 - 280 of the 1,2,4-Trihydroxybenzene package from the December 2023 meeting (https://www.cir-safety.org/sites/default/files/1,2,4-Trihydroxybenzene.pdf).

Please be prepared to discuss the Council's comments at the June meeting.

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: May 21, 2024

SUBJECT: Draft Final Report: Safety Assessment of 1,2,4-Trihydroxybenzene as Used in Cosmetics (draft prepared for the June 2024 meeting)

The Personal Care Products Council respectfully submits the following comments on the draft final report, Safety Assessment of 1,2,4-Trihydroxybenzene as Used in Cosmetics.

Key Issues

Genotoxicity – The expert opinion/analysis concerning the genotoxicity potential of 1,2,4-Trihydroxybenzene by Drs. Barry Halliwell and Marilyn Aardema should be considered for addition to the CIR report. Expert opinions provided as unpublished data should not always be dismissed and not included in CIR reports. The Expert Panel should determine whether the expert opinion/analysis should be cited in a CIR report.

Margin of Safety; Summary - Providing the concentration in the first paragraph of the Margin of Safety section (and later in the Summary) is not sufficient. It should also indicate that the concentration refers to use in a hair dye.

Margin of Safety - Please provide the reference for the 90-day study and state the effect at the LOAEL (increased spleen weights).

Discussion – If a MoE or MoS calculation is completed in a CIR report, it should be mentioned in the Discussion. It would be helpful to note the differences in results in genotoxicity studies with and without measures to control oxidation of 1,2,4-Trihydroxybenzene.

Additional Considerations

Impurities – It would be helpful if the same name was used for an impurity, e.g., first paragraph tetrahydroxybenzene, second paragraph benzene-1,2,4-tetraol (these are 2 names for the same substance).

Cosmetic Use – It should be stated that the specialized packaging is used for hair dye formulations containing 1,2,4-Trihydroxybenzene.

Cosmetic Use – When discussing the European regulations for 1,2,4-Trihydroxybenzene, it would be helpful to add that because of the ban on animal testing of cosmetics and cosmetic ingredients in Europe, the SCCS was not able to review the recent in vivo genotoxicity data.

Genotoxicity – Please correct; "1,2,4-Trihydroxybenz[e]ne" (add "e")

Immunomodulatory Effects – In what group of mice was the "lymph node weight significantly lowered" with LNC not affected? It does not make sense for this to be the 1,2,4-Trihydroxybenzene treated mice that were sensitized to DNCB as "DNCB-sensitized cell proliferation was increased by approximately 2-fold by supplementation of 1,2,4-Trihydroxybenzene" (which would increase lymph node weight and LNC number).

Commitment & Credibility since 1976

Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Priya Cherian, M.S., Senior Scientific Writer/Analyst, CIR
Date: May 24, 2024
Subject: Wave 2 – Data on Yeast-Derived Ingredients and Council Comments

Attached is summary human dermal irritation and sensitization data (*data_Yeast_Wave2_062024*) on a Yeast Extract derived from *Torulaspora delbrueckii* (0.12% in water). The test substance was considered to be non-irritating and non-sensitizing. With the inclusion of this information, the following *Torulaspora delbrueckii*-derived ingredients now have both dermal sensitization data and food use/GRAS/QPS status data:

Hydrolyzed Torulaspora Delbrueckii Extract
Torulaspora Delbrueckii Extract
Torulaspora Delbrueckii Ferment

In addition, comments were received from Council (*PCPCcomments_Yeast_Wave2_062024*). These comments have been included herein.

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: May 21, 2024

SUBJECT: Draft Final Report: Safety Assessment of Yeast-Derived Ingredients as Used in Cosmetics (draft prepared for the June 2024 meeting)

The Personal Care Products Council respectfully submits the following comments on the draft final report, Safety Assessment of Yeast-Derived Ingredients as Used in Cosmetics.

Key Issue

In the Abstract and Discussion, it is not clear why it is necessary to specifically mention pesticide residues. The only study that measured pesticide residues found that they were below the limits of quantification.

The abstract also states: "The Panel noted that elevated levels of heavy metals and pesticide residues may be present in yeast derived ingredients..." This is not accurate as there are no studies in the report that show elevated levels of heavy metals or pesticide residues.

Additional Considerations

Dermal Irritation and Sensitization – Please correct: "No irritation [w]as observed..." (add "w")

Discussion – Please replace "formulated" in the following with "manufactured": "if formulated using a species of yeast included in this report..."

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: May 16, 2024

SUBJECT: Yeast Extract derived from *Torulaspora delbrueckii*

Anonymous. 2011. Summary information dermal irritation and sensitization – Yeast Extract made from *Torulaspora delbrueckii*.

Summary Dermal Irritation and Sensitization – Yeast Extract made from *Torulaspora delbrueckii*

Dermal Irritation

Single insult patch test completed in April 2011 in 10 volunteers

Test material: Aqueous Yeast Extract made from *Torulaspora delbrueckii* tested at 0.12% in water

Results: non-irritant

Dermal Sensitization

Human repeated insult patch test completed in July 2011 in 100 volunteers

Test material: Aqueous Yeast Extract made from *Torulaspora delbrueckii* tested at 0.12% in water

Results: non-irritant and non-sensitizing